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ROOT FORMATION AND FLOWERING OF DAHLIA CUTTINGS WHEN SUBJECTED TO DIFFERENT DAY LENGTHS[1]

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(WITH SIX FIGURES)

Introduction

Dahlias propagated from cuttings during late summer and fall vary in response as the days become shorter. Cuttings taken in August produce normal fibrous roots and become plants of good size before flowering. Cuttings taken in late September and October produce storage roots at the sacrifice of fibrous roots, and flower when only a few inches high. Often no roots at all are formed on cuttings taken in October, but the basal ends of the stems serve as storage centers. Occasionally the buds along the stem become storage organs, swelling and resembling tubers. When six hours of extra light are used to supplement the daylight, the plants produce normal fibrous roots and are slow to flower. GARNER and ALLARD (5), ARTHUR and GUTHRIE (1), and ARTHUR (2) have reported many plant species which flower more readily in short day lengths than in long days. GARNER and ALLARD reported one variety of dahlia, John Ehlich, which flowered on July 8 in the 10-hour day as compared with September 27 for the check grown in the natural day length. They also reported that storage roots and tuber formation

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

of several species of plants are affected by day length. Two unnamed dahlia varieties did not show the usual "tuber" formation when grown in lengthened illumination periods. A bean (*Phaseolus multiflora* Willd.) and a yam (*Dioscorea alata*) formed larger storage roots in 10- to 12-hour days than in the full day length of summer. McCormick potatoes did not tuberize with extra illumination added to the usual day length of summer. Artichokes (*Helianthus tuberosus*) in short days formed mature looking tubers in contrast with the slender, immature types of the long day plants.

The purpose of this paper is to report the response of cuttings from several varieties of dahlia when subjected to different day lengths.

Methods and materials

During the late summer and fall of 1926, cuttings were taken at one-week intervals from dahlias growing on the Institute grounds and were planted in the greenhouse in sand or mixtures of peat moss and sand. The material was generally young shoots coming from the main stem rather than from the ends of large active branches. In this way it was possible to get material free from flower buds. After cuttings were rooted, they were potted in good garden soil and grown either in a greenhouse receiving normal fall light or in an adjoining greenhouse where they were given extra light. The extra light was given from sunset to 12 o'clock midnight by a 1500-watt nitrogen bulb.

Seedlings which were also included in the experiment were from a promiscuous collection of seeds planted on November 3. About 1000 seedlings resulted, and from these the best plants were selected for experimental work.

During 1928 the plants used came, for the most part, from select material purchased from Downs Dahlia Farm, Clayton, New Jersey, and from Fisher and Masson, Trenton, New Jersey. The storage roots were placed in a peat moss medium, and cuttings were made as the shoots appeared above the surface. These cuttings were placed directly into pots containing a mixture of peat moss and garden loam. Later the established plants were repotted in fertilized soil and subjected to different amounts of light. One lot was grown outdoors as a check; a second lot by the check was given 7 hours of

daylight and the remainder of the day in the dark room; a third lot by the check was given 9 hours of daylight and the remainder of the day in the dark room.

Nitrate tests were made with a solution of diphenylamin in sulphuric acid (0.1 gm. of diphenylamin to 10 cc. of 75 per cent H_2SO_4).

FIG. 1.—*Dahlia variabilis* (Jersey's Beauty) from cuttings, August 26 to November 15

Hydrogen-ion measurements were made with a quinhydrone apparatus. Stems and leaves were cut into small pieces and then crushed in a mortar. Some of the crushed plant material, as well as the juice, was transferred to the electrode chamber. It will be noted that the sample thus measured contained both tissue and its expressed juice.

Results and discussion

EXPERIMENTS WITH FALL CUTTINGS.—Cuttings taken on August 26 from dahlia varieties, such as Jersey's Beauty, Mrs. I. de ver Warner, or Peg-O-My-Heart, formed normal fibrous roots by Sep-

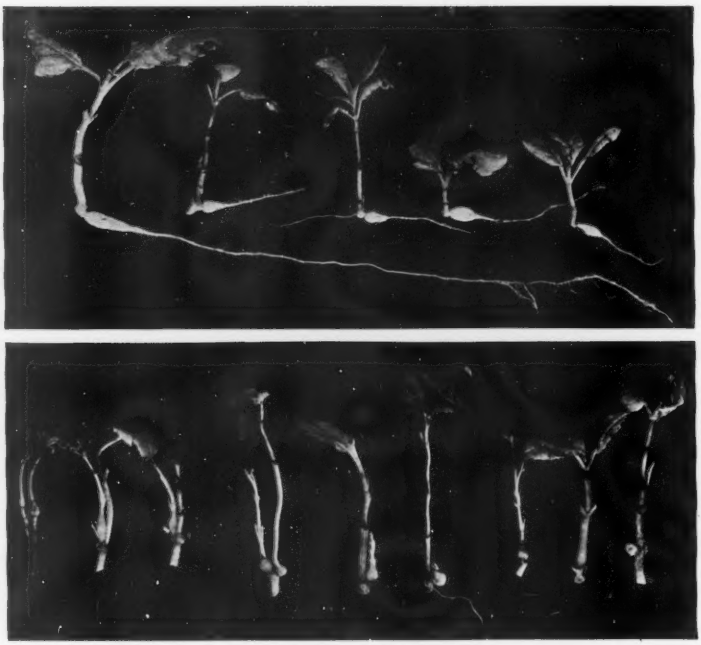

FIG. 2.—*Dahlia variabilis* (Jersey's Beauty) cuttings, October to December, as they appeared when taken from rooting medium; note almost complete absence of fibrous roots; upper figures, main roots developed are storage organs; lower figures, left, storage in base of new shoot arising after cuttings were made; middle, basal buds form storage organs comparable with tubers; right, portions of cutting, as stem and leaf petiole, serve as storage organs.

tember 17 and many flowered by November 15. They did not flower uniformly, but continued up to December 15. At the time of flowering the plants varied from 1 to 3 feet in height (fig. 1). Five plants given 6 hours of extra light had not flowered by February 7, and neither had they formed storage organs. Fibrous roots and large tops

developed at the expense of storage roots. The extra light plants ranged from 3 to 4 feet in height, while checks varied from 1 to 3 feet in height.

Cuttings taken from the same varieties on September 26 produced a few fibrous roots, but there was a tendency to produce storage roots while still in the rooting medium. Flower buds were formed when the plants were 10–12 inches high. Flowering and the formation of storage roots took place concurrently from October to December.

Cuttings taken from October 15 to October 28 developed storage roots, as a rule, instead of the normal fibrous roots characteristic for early cuttings. In many instances no roots formed, but various parts of the stems became storage organs (fig. 2). In some cases buds developed into organs resembling bulbs or tubers; in others the stems became storage centers; and in still others remaining portions of leaf petioles were enlarged. Where roots formed, as a rule they were few and mostly of the storage type. A few cuttings without roots flowered while in the rooting medium.

EXPERIMENTS WITH SEEDLINGS.—Approximately 100 seedlings grown from seeds planted November 3 were treated with normal and extra light. Much variation was evident, but the prevailing tendency was for the plants to flower and form storage roots in the short winter days. Plants given extra light formed fibrous roots only, and did not flower during the course of the experiment (fig. 3). Seedlings first developed a fibrous root system from the hypocotyl, and when the plants were 4 or 5 inches high, special roots appeared just below the cotyledons. These roots had a fleshy appearance and quickly developed into storage organs during the short days.

EXPERIMENTS WITH SPRING AND SUMMER CUTTINGS.—On June 8, thirty Jersey's Beauty dahlia plants, half from cuttings and half with tubers attached, were divided into three lots of ten each, and given the following treatment:

Lot 1, grown outdoors in normal day length.

Lot 2, grown outdoors for 9 hours each day and the remainder of the time in the dark room.

Lot 3, grown outdoors for 7 hours each day and the remainder of the time in the dark room.

FIG. 3.—Seedling dahlias: *A*, grown from November 3 to February 7; left, plants receiving normal light during winter months flowered when only a few inches high; right, same as those on left in the beginning, but given extra illumination from sundown to midnight: *B*, grown from November 3 to January 4; two large plants with fibrous roots given extra light from sundown to midnight; small dahlias with storage roots received usual light of November and December: note that extra light most completely eliminated formation of storage roots, while short day plants, although only a few inches high, produced unusually large storage roots.

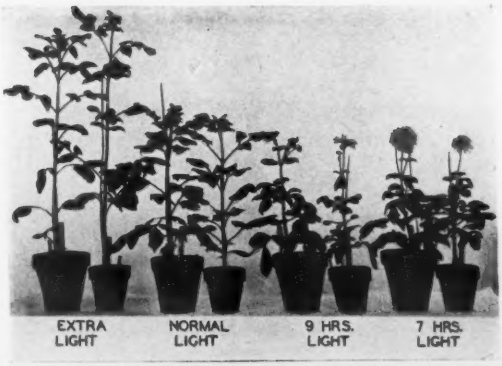

FIG. 4.—Top, *Dahlia variabilis* (Jersey's Beauty), June 8 to July 17, showing effect of length of day on top growth and flowering; plants in large pots had old storage root attached; plants in small pots were grown from cuttings; first flower buds occurred in short day plants on June 26, and on long day plants August 6; all short day plants had flowered by July 17; normal light plants started to flower August 20. Middle, root systems of plants from lots photographed in top row; extra light plants were omitted, otherwise the order is as appears in top row; photographed August 6. Note heavy root storage in short day plants; normal light plants developed fibrous roots with only a slight tendency to produce storage roots; when storage roots started, they were elongated and unlike the storage roots of short day plants. Below, Mrs. I. de ver Warner dahlias showing effect of length of day on roots and tops; labeled as in top row, started June 8 and photographed August 6; this variety responds almost exactly like Jersey's Beauty; in extra light plants where there is a tendency to form "tubers" there is great contrast to the short ball-like storage roots of short day plants.

Table I shows the effect of length of day on the type of roots produced, time of flowering, and the supply of nitrates present. Lots 2 and 3 were very much alike throughout, varying only in size. The normal day plants produced many more leaves and became larger in general than short day plants. Microchemical tests showed a very high supply of nitrates in the leaves of short day plants, while very little appeared in the normal day plants. The hydrogen-ion concentration of expressed sap from stems or leaves deviated only slightly

TABLE I

EFFECT OF LENGTH OF DAY ON TYPE OF ROOTS AND TIME OF FLOWERING OF JERSEY'S BEAUTY DAHLIA; DURATION OF EXPERIMENT JUNE 8 TO AUGUST 6, 1928

LIGHT TREATMENT	NO. OF PLANTS USED		DATE OF FIRST VISIBLE FLOWER BUDS	NO. OF PLANTS FLOWERING ON JULY 17	AVERAGE HEIGHT		TYPE OF ROOTS FORMED	NITRATES (MICROCHEMICAL TESTS)
	Cuttings	With old "tubers"			Cuttings	With old "tubers"		
Lot 1, normal day length.	5	5	August 6	0*	2′6″	3′	Fibrous roots with slight tendency to form storage roots	Nitrates low in leaves and stems
Lot 2, 9 hours of daylight.	5	5	June 28	10	1′6″	1′8″	Storage roots with comparatively few fibrous roots	Nitrates abundant in leaves and stems
Lot 3, 7 hours of daylight.	5	5	June 26	10	1′4″	1′6″	Storage roots with comparatively few fibrous roots	Nitrates abundant in leaves and stems

* A few plants remaining after the experiment was discontinued showed flower buds on August 31.

from pH 5.4 in plants of all lots. The normal light plants developed mostly fibrous roots with only an indication of storage, while both the 7-hour and 9-hour plants had large storage roots. Where storage roots had started on normal day plants they were slender, with long necks, whereas those on short day plants were short and rounded like potato tubers (fig. 4).

In order to find whether the presence of stored food material influenced the type of root system or the time of flowering, five plants in each lot were grown with old "tubers" attached. The response was essentially like that of cuttings (fig. 5).

In addition to Jersey's Beauty, six other named varieties have been used in experiments. Of these, four varieties (Mrs. I. de ver

Warner, Trentonian, F.T.D., and Insulinda) formed storage roots and flowered only when given short day treatment. Table I, showing data for Jersey's Beauty, could almost exactly be duplicated for Mrs. I. de ver Warner. The main difference was a slightly earlier bud development in Warner plants. Arthur (pompon), Esther R. Holmes, Maid of Watts, and Summer Red were indifferent to length of day in respect to flowering, but storage roots were favored by short days (fig. 6).

There are many varieties of dahlia that flower throughout the long days of summer. The pompon types, particularly, are known for this characteristic. Fig. 6 shows two pompons (Arthur) which flowered in both long and short days. They show, however, a great difference in root storage. The photograph showing roots was taken on August 8, after the days were beginning to shorten. Note that the long day plant has many fibrous roots, but carbohydrate storage had started. The short day plant, however, has much heavier storage. In this case it is clear that carbohydrate accumulation is independent of flowering, but is controlled by the length of day. Also flowering is independent of storage root formation, for in the long day plant, flowering had been accomplished before storage had started in the roots. In types like Jersey's Beauty, flowering and the development of storage roots took place concurrently in the short days. Long day plants could form neither storage organs nor flowers (fig. 5). It so happens that the same factor, the length of day, controls both responses at the same time in this type of dahlia, although root storage and flowering are probably not correlated in a causal way. This supposition is strengthened by the fact that plants grown with old storage roots attached responded to day length almost exactly as did cuttings (fig. 6). "Tubers" attached to the pompon dahlias did not change their flowering response, as these plants flowered under all conditions.

Temperature was not controlled in any of the experiments with dahlias. Possibly both light and temperature could be determining factors. In the case of potatoes, GARNER and ALLARD (5) showed that tuberization could be completely eliminated by extra illumination. BUSHNELL (3), SMITH (7), FITCH (4), and others completely eliminated tuberization of potatoes by keeping the temperature up

FIG. 5.—*Dahlia variabilis* (Jersey's Beauty) plants with old storage root attached, June 8 to August 6; left to right, normal light, 9 hours' light, and 7 hours' light: note that short day plants flower and produce storage roots the same as do plants grown from cuttings; there is no noticeable influence from the old storage root (indicated by black cross).

FIG. 6.—*Dahlia variabilis* [Arthur (pompon)]: *A*, plants from cuttings, June 8 to July 16; plant on left received normal light; plant on right received 9 hours of light; this variety flowers on both long and short day illumination; *B*, plants same as those photographed in *A*, but grown from June 8 to August 6: note that root types are same as in other varieties, although flowering is irrespective of light.

to 29° C., while plants at 20° C. tuberized heavily. This suggests that work ought to be done in which both light and temperature are taken into consideration.

The effect of light on cuttings while they are in the rooting medium has long been a subject under discussion. The point of interest has usually been the effect of light intensity. In case of dahlia both light intensity and duration are important. Vegetative growth is so limited by short light duration that few or no fibrous roots can be produced. Such roots as can grow are storage in nature. Many variations from normal storage roots have been noted. Old stems become modified at points where carbohydrates accumulate, and the lowest buds develop into tuber-like organs (fig. 2). Leaves placed in the rooting medium often formed roots, but whether they rooted or not, the basal end of the petiole became enlarged and took the place of storage tissue. Cuttings grown earlier in the season, when the days were longer, had no such tendency toward developing storage organs. Normal fibrous roots were the rule. There is doubtless a certain day length where both vegetative growth and storage can proceed at an even rate. On either side of this particular duration one would have an advantage over the other.

It is of interest to note that nitrates accumulate in the short day plants (table I). NIGHTINGALE (6) found that *Salvia*, buckwheat, and soy beans illuminated for only 7 hours each day had a higher percentage of nitrates and carbohydrates than the long day plants. He states that carbohydrates accumulate in the short day plants, presumably because there is relatively little utilization of them in the synthesis of nitrates to other forms of nitrogen. Even though growth is checked, if the plant remains in good condition, carbohydrates will continue to be manufactured and nitrates to be absorbed. It seems reasonable to suppose that when any growth process is interfered with, the utilization of the two main building materials, carbohydrates and nitrates, will correspondingly decrease, thus permitting their accumulation. In the case of the dahlia, a 7- or 9-hour day quickly brings vegetative growth to an end and flowering is accomplished. The plant remains in good condition thereafter for a considerable period of time, continuing to manufacture carbohydrates and absorb nitrates. As these two substances accumulate, the plants take on the appearance of approaching dormancy. They

do pass, in time, into complete dormancy and lie for two or more months before they are able to grow. If they are changed early enough from short to long day, dormancy is prevented and vegetative growth starts.

Summary

1. Length of day determines the type of root system formed by cuttings for 6 varieties of dahlia, heavy root storage being correlated with a short day, and a fibrous root system being correlated with a long day.

2. Flowering was found to be independent of storage root formation, although certain varieties, such as Jersey's Beauty and Mrs. I. de ver Warner, flowered and formed storage roots concurrently on a short day.

3. Certain varieties, such as Arthur and Summer Red, flowered independently of day length; while others, such as Jersey's Beauty and Warner, flowered only on a short day.

4. Cuttings taken during late September and October developed various types of storage organs along the stem without forming either typical storage roots or fibrous roots.

5. Nitrates accumulated in the leaves and stems of short day plants, but were absent or present in only small amounts in long day plants.

6. The hydrogen-ion concentration of stems or leaves of long and short day plants varied only slightly from pH 5.4.

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MOSAIC DISEASE OF TOBACCO[1]

C. G. VINSON AND A. W. PETRE

Introduction

BEIJERINCK (3) reported working with an infectious juice expressed from plants with mosaic disease. He obtained a precipitate with alcohol from the juice. This precipitate, after drying at 40° C., retained its infectivity. HEINTZEL (8) obtained the same results as BEIJERINCK. ALLARD (1), VINSON (16), and SMITH (15) have also reported the use of alcohol in precipitating the virus from juice of diseased plants. ALLARD (1) has shown that an active fraction can be adsorbed from juice of diseased plants by talc and aluminium hydroxide. He also obtained a glycerin extract from dried, ground leaf material which, after filtering, appeared to contain more of the original activity than the residue. VINSON (16), in addition to corroborating the observations on precipitation by alcohol, reported analogous behavior with acetone, ammonium sulphate, and safranin. BREWER, KRAYBILL, and GARDNER (4) reported activity in a colorless solution obtained by adsorbing the virus on charcoal, washing out the coloring matter, and then freeing the virus.

The biological reaction is the only test available, so far as known, for determining the presence of the virus. No distinction can be made as to the relative virus concentrations when the methods of inoculation in general use are employed. MCKINNEY (12) has called attention to the desirability of a method for determining virus concentrations, and has devised a method which detects considerable differences in virus concentration. The method of HOLMES (9), based on the introduction of a small dose, detects differences in virus concentration within more restricted limits.

This paper gives in detail the results obtained in removing the virus from infectious juice by methods previously described (16), and also reports further progress in freeing the virus of accompany-

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

ing solids. We submit evidence, based on measurements obtained by an adaptation of HOLMES' technique, that the virus of mosaic disease of tobacco can be freed of 90 per cent of the non-active solids of the juice with no apparent loss in infective power.

Preliminary work

Only diseased tomato plants were at first available. Leaves and tips of these plants were collected from the field. Part of this material was treated with ether (5) in the fresh condition, and the cell sap expressed at once with a hydraulic press. The other part of the plant material collected was placed in a cold room and allowed to freeze. It was then thawed and the cell sap expressed. Both lots of juice proved to be infectious.

On concentration in vacuo the juice frothed badly, especially that from the etherized plants. Caprylic alcohol and diphenyl ether were successfully used as foam breakers. A few drops of either of these substances reduced the tendency to froth considerably, but after their use the juice became inactive, even though the temperature of the water bath was never allowed to rise above 50° C. It was found, however, that juice from frozen plants could be concentrated in vacuo without troublesome frothing, the flask requiring close attention only when boiling began.

An attempt was made to remove some of the solutes present in the concentrated juice by electrodialysis. The first solution electrodialyzed was one prepared by concentrating 2300 cc. of juice from frozen tomato plants to 150 cc. To the 150 cc. of concentrate 20 per cent, by volume, of ethyl alcohol was added as a preservative. This solution was infectious. Its pH was 4.76. In the electrodialysis work a Y tube was used, and the ends of the two arms covered with linen cloth impregnated with collodion. The arms on the Y were inserted into two separate small beakers of distilled water containing a trace of magnesium sulphate as electrolyte. Platinum electrodes were inserted in the beakers, consequently the electrodes did not come in contact with the concentrated juice. The Y tube was then filled up to the stem with the concentrated juice. The dialysis was allowed to run for about 18 hours, the current being about 10 milliamperes at 20 volts. When the experiment was terminated, samples were

taken from both of the beakers and both arms of the Y tube. That from the arm of the tube projecting into the beaker which contained the negative electrode was infectious. The other samples were not infectious. The experiment was repeated on a second portion of the same sample, and the same results were obtained. This indicated that the active principle, under certain conditions, carried an electric charge.

Experimental work

Preparation of material

Tobacco plants were used in the following experiments. The original stock of virus was obtained from Professor James Johnson,

TABLE I

Data showing most of virus is obtained in juice expressed from tissues first frozen, then thawed

Dilution	Expressed juice 700 cc.		First aqueous extract of press cake, 600 cc.		Second aqueous extract of press cake, 604 cc.		Third aqueous extract of press cake, 523 cc.	
	No. of plants inoculated	No. diseased	No. of plants inoculated	No. diseased	No. of plants inoculated	No. diseased	No. of plants inoculated	No. diseased
Undiluted.....	10	10	10	8	10	7	10	6
1/250.....	10	6	.....	.....	.....	.....	10	0
1/500.....	10	6	10	1	10	1	.....	.....
1/1000.....	10	6	10	2	.....	.....	.....	.....
1/5000.....	10	4	.....	.....	.....	.....	.....	.....
1/10000.....	10	2	.....	.....	.....	.....	.....	.....

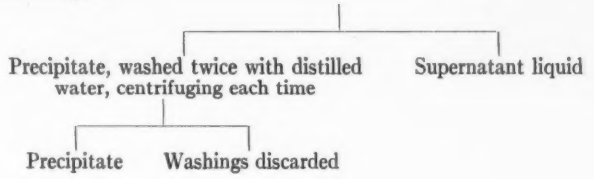
Department of Plant Pathology, University of Wisconsin. This virus gave the "marked mottling, malformation and stunting" characteristic of the mosaic disease of tobacco as found in the field. The juice samples on which our observations have been made were obtained from frozen diseased leaves, or from leaves and stalks after thawing at room temperature. The juice was pressed out of the thawed material (without grinding) at 500 pounds per square inch, then centrifuged for 10 minutes at 1500 r.p.m. This gives, in the centrifuge used, a centrifugal force about 600 times that of gravity. The juice obtained is brown, and free from large suspended particles. That obtained only from leaves contains about 22 per cent of the leaf solids and 12.5 per cent of the leaf nitrogen. That the juice obtained

by this method from diseased plants contains the greater portion of the virus is shown by the infectivity of the expressed juice, as compared with the aqueous extracts of the ground press cake. Results of tests of juice and extracts are given in table I.

Precipitation of virus with an aqueous solution of safranin

The results in electrodialysis, and the fact that Allard (1) was able to remove the virus from solution with talc, gave evidence that the virus possessed some properties characteristic of colloids. It was natural, therefore, to expect that certain substances which could neutralize the charge or affect the water relationship of the active principle might throw it out of solution. Solutions of certain dyes were therefore added to samples of juice from diseased tobacco plants. Salts of acid dyes such as crystal ponceau and rose bengal gave a precipitate when added to juice from diseased plants. That produced by rose bengal settled rapidly, but when the supernatant liquid was tested it was found to be active. Solutions of basic dyes such as Bismarck brown and safranin were tried. Bismarck brown gave a precipitate which settled rapidly, but the supernatant liquid retained activity. Ten cc. of a 1 per cent safranin solution when added to 50 cc. of juice from diseased plants gave a precipitate which flocculated and settled very slowly. The supernatant liquid was, according to our determinations, almost free from virus. Safranin, therefore, was the only one of these dyes found capable of precipitating the virus almost completely. It is to be expected, however, that other azine dyes will also precipitate the virus.

The safranin precipitate was obtained according to the following scheme. Juice from diseased plants was treated with a 1 per cent aqueous solution of safranin in the proportion 10 to 50 cc. of the juice, then placed in the ice box for several hours, usually overnight, and centrifuged:



FREEING VIRUS FROM SAFRANIN PRECIPITATE

Safranin being a weak base, it was thought that by adding a small amount of acid or a small amount of stronger base to a suspension of the safranin precipitate, the virus might be set free. Table II gives the results obtained in preliminary attempts to free the virus from the precipitate by acid and alkali treatments. As indi-

TABLE II

ATTEMPTS TO FREE VIRUS FROM SAFRANIN PRECIPITATE BY ACID AND ALKALI TREATMENT

EXPERIMENT NO.*	PREPARATION	TREATMENT	NO. OF PLANTS INOCULATED	NO. DISEASED
1..	(a) Precipitate suspended in 0.5 per cent agar solution	None	10	1
	(b) 15 cc. of suspended precipitate	+3 cc. N/14 HCl	5	1
	(c) 15 cc. of suspended precipitate	+3 cc. N/14 NaOH	5	0
	(d) 30 cc. of supernatant liquid	+3 cc. N/14 HCl	5	0
	(e) 30 cc. of supernatant liquid	+3 cc. N/14 NaOH	5	0
2..	(a) Precipitate suspended in 0.5 per cent agar solution	None	10	1
	(b) 25 cc. of suspended precipitate	+7 cc. N/10 acetic acid	10	0
	(c) 25 cc. of suspended precipitate	+5 cc. N/10 Na_2CO_3 solution	10	0
	(d) Supernatant liquid	None	10	0
	(e) Supernatant liquid	Diluted 1 to 10	10	0
3..	(a) Precipitate suspended in 0.5 per cent agar solution	None	10	1
	(b) 25 cc. of suspended precipitate	+6 cc. N/10 acetic acid in cold	10	2
	(c) 25 cc. of suspended precipitate	+5 cc. N/10 Na_2CO_3 in cold	10	4
	(d) Supernatant liquid	None	10	0
	(e) Supernatant liquid	Diluted 1 to 10	10	0
4..	(a) Precipitate suspended in dilute agar	None	10	1
	(b) 25 cc. of suspended precipitate	+5 cc. N/10 acetic in cold, pH 4.8	10	2
	(c) 25 cc. of suspended precipitate	+5 cc. N/10 Na_2CO_3 in cold, pH 7.2	10	2
	(d) Supernatant liquid	None	10	0
	(e) Supernatant liquid	Diluted 1 to 10	10	1
5..	(a) Precipitate suspended in aqueous trypsin extract and let stand over-night in ice box	None	10	5
	(b) Precipitate suspended in aqueous pepsin solution 3 hours	None	10	5

* The blanks used in these experiments were all healthy at the end of the respective tests.

cated in the table, these attempts were not successful. The dilutions of the supernatant liquid, 1 to 10, were made in order to reduce the concentration of the safranin to the point where it was known that it would not inhibit the activity of the virus. It was found by trial that juice from diseased plants treated at the rate of 1 cc. of a 1 per cent aqueous solution of safranin to 50 cc. of juice remains infectious. It is necessary to add an excess of the safranin solution to complete the precipitation.

ROBERTSON (14), HOLZBERG (10), and MARSTON (11) have shown that safranin forms a rather insoluble precipitate with proteoclastic enzymes. The safranin precipitate of the virus was treated, therefore, with a trypsin and also with a pepsin solution. By means of these treatments the virus was apparently displaced from its union with the safranin (see experiment 5, table II).

A means of removing the safranin from the safranin-virus precipitate, without inactivating the virus as it was set free, was then sought. After some preliminary tests it was found that picric acid forms a very insoluble precipitate with safranin. When 2 cc. of a saturated aqueous solution of picric acid is added to 25 cc. of a 0.16 per cent aqueous safranin solution, the precipitation is practically complete.

In attempts to free the virus from the safranin precipitate with picric acid, the following procedure was followed:

To 50 cc. of juice from diseased plants was added 15 cc. of a 1 per cent aqueous safranin solution; solution then placed in ice box several hours, preferably overnight, and centrifuged

- Safranin precipitate washed once with 70 cc. distilled water and centrifuged
  - Safranin precipitate suspended in 25 cc. of distilled water; a saturated aqueous solution of picric acid added; let stand for some hours, then centrifuged
    - Supernatant liquid used for inoculating plants
    - Precipitate discarded
  - Washings discarded
- Supernatant liquid decanted, and to 25 cc. of the supernatant liquid 2 cc. of a saturated aqueous solution of picric acid added, then centrifuged
  - Supernatant liquid, free from safranin, used for inoculating plants
  - Precipitate discarded

Table III gives the results obtained in freeing the safranin-virus precipitate from safranin. Experiment 5 indicates that the picric

TABLE III

LIBERATION OF VIRUS FROM SAFRANIN PRECIPITATE BY MEANS OF PICRIC ACID

EXPERIMENT NO.	PREPARATION	pH OF SOLUTION	NO. OF PLANTS INOCULATED	NO. DISEASED
1..	(a) Safranin precipitate suspended in 25 cc. distilled water and 2 cc. saturated aqueous solution of picric acid added; after standing, solution centrifuged and supernatant liquid used for inoculating plants	3.9	10	4
	(b) 25 cc. of supernatant liquid from virus-safranin precipitate treated with 2 cc. of saturated aqueous solution of picric acid, centrifuged, and supernatant liquid, free of safranin, used for inoculating plants	5.5	10	0
2..	(a) Safranin-virus precipitate suspended in 25 cc. distilled water and 2 cc. saturated aqueous solution of picric acid added; after standing, solution centrifuged and supernatant liquid used for inoculating plants	4.3	10	3
	(b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1..	5.3	10	0
3..	(a) Safranin-virus precipitate suspended in 25 cc. distilled water and 4 cc. saturated aqueous solution of picric acid and 1.2 cc. N/10 Na_2CO_3 solution added; after standing, solution centrifuged and supernatant liquid used for inoculating plants	3.7	10	3
	(b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1..	5.2	10	0
4..	(a) Safranin-virus precipitate suspended in 25 cc. distilled water and 4 cc. picric acid and 2 cc. N/10 Na_2CO_3 solution added; after standing, centrifuged, and supernatant liquid used for inoculating plants	6.6	10	9
	(b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1	5.3	10	2
5..	(a) To 25 cc. of diseased juice was added 2 cc. of saturated aqueous solution of picric acid, and solution used for inoculating plants	5.3	10	10

acid concentration used to remove safranin from the supernatant liquid of the virus-safranin precipitate does not affect the activity of the virus appreciably.

Removal of safranin from safranin-virus precipitate by means of amyl alcohol

On removal of the dye with normal amyl alcohol from the suspension of the safranin-virus precipitate, following dispersion in acid solution, activity is recovered in the aqueous layer. Hydrochloric, nitric, acetic, and oxalic acids have been used successfully to effect the dispersion of the precipitate and release the virus. When the volume of water used to disperse the precipitate is twice the volume of the juice from which the precipitate is obtained, the addition of acid produces a clear transparent solution. Under these conditions the liberation of the virus from the dye appears to be optimal. The aqueous solution obtained following amyl alcohol washing is brown in color, resembling in appearance the original juice from diseased plants. A precipitate separates at the water-amyl alcohol interface; this precipitate is also active. The safranin-virus precipitate was obtained by adding 12 cc. of a 1 per cent aqueous safranin solution to 50 cc. of juice from diseased plants. Table IV gives the results obtained.

TABLE IV

Liberation of virus from safranin precipitate by extracting safranin with amyl alcohol

Experiment no.	Preparation	No. of plants inoculated	No. diseased
1..	(a) Aqueous layer, 50 cc. in volume, pH 5.8–6.0, after removing safranin from aqueous suspension of precipitate with amyl alcohol	10	10
	(b) Suspension of interfacial precipitate formed on removal of safranin with amyl alcohol	10	7
2..	(a) Safranin-virus precipitate suspended in 50 cc. H_2O and 1 cc. N. HCl added; pH of resulting solution 2.9; safranin removed with amyl alcohol; aqueous layer, safranin free, but not neutralized	10	9
	(b) Same as 2(a), but neutralized	10	10
	(c) Same as in experiment 1(b)	10	10
3..	(a) Safranin-virus precipitate suspended in 50 cc. H_2O and 0.35 cc. N/1 HCl added; pH of resulting solution 3.1–3.2; aqueous layer, safranin free, but not neutralized	10	10
	(b) Same as experiment 3(a), but neutralized	10	10
	(c) Same as in experiment 1(b)	10	10
	(d) Supernatant liquid from safranin precipitate after removing safranin with amyl alcohol	10	3

To discover whether the conditions for safranin precipitation would be more favorable in an alkaline medium, samples of juice from diseased plants were brought to a hydrogen-ion concentration of pH 7.2 by the addition of saturated calcium hydroxide solution. Twelve cc. of a 1 per cent safranin solution was then added to 50 cc. of the treated juice. The precipitate was removed by centrifuging,

TABLE V

PRECIPITATING THE VIRUS WITH SAFRANIN FROM JUICE AT ALKALINE REACTION, THEN REMOVING THE DYE FROM PRECIPITATE AND SUPERNATANT LIQUID WITH AMYL ALCOHOL

EXPERIMENT NO.	PREPARATION	NO. OF PLANTS INOCULATED	NO. DISEASED
1..	(a) Safranin-virus precipitate suspended in 65 cc. H_2O; pH of resulting suspension 7.7; aqueous layer, following removal of safranin, with amyl alcohol	10	10
	(b) Suspension of interfacial precipitate after washing twice with water	10	9
2..	(a) Virus-safranin precipitate suspended in 50 cc. water, 1 cc. N/1 HCl added; pH of resulting suspension 3.0; safranin free aqueous layer, not neutralized	10	10
	(b) Same as 2(a), but neutralized	10	10
	(c) Same as in experiment 1(b)	10	10
3..	(a) Safranin-virus precipitate suspended in 50 cc. water and brought to pH 3.6 by adding N/1 HCl; aqueous layer, following removal of safranin with amyl alcohol, not neutralized	10	10
	(b) Same as in experiment 3(a), but neutralized	10	10
	(c) Same as in experiment 1(b)	10	10
	(d) Supernatant liquid from safranin-virus precipitate after removal of safranin with amyl alcohol	10	2
	(e) Untreated diseased juice	10	9
	(f) Diseased juice diluted 1 to 1000	10	1

then washed with 50 cc. of distilled water. Table V gives the results obtained on removing the dye from the precipitate and also the supernatant liquid with amyl alcohol.

SALTING OUT EXPERIMENTS ON VIRUS OF TOBACCO MOSAIC

Salting out experiments were tried after it was found that an aqueous solution of safranin precipitated the virus, and also that under certain conditions the virus apparently will migrate in an electric field.

TABLE VI

SALTING OUT THE VIRUS FROM JUICE OF DISEASED PLANTS

EXPERIMENT NO.	PREPARATION	NO. OF PLANTS INOCULATED	NO. DISEASED
1...	(a) 34 gm. $(NH_4)_2SO_4$ added to 50 cc. juice from diseased plants at room temperature, centrifuged; precipitate dissolved in 20 cc. distilled water, and solution used for inoculating plants	5	2
	(b) Supernatant liquid diluted with five volumes of distilled water	5	0
2...	(a) 17 gm. $(NH_4)_2SO_4$ added to 25 cc. juice from diseased plants at about 0° C. in cold; after agitating, precipitate filtered off and dissolved in 15 cc. distilled water, and solution used for inoculating plants	5	4
	(b) 5 cc. filtrate made up to 50 cc. and solution used for inoculating plants	5	0
3...	(a) 16.5 gm. $(NH_4)_2SO_4$ added to 25 cc. juice from diseased plants at about 0° C. in cold; after agitating, precipitate filtered off and dissolved in 20 cc. distilled water and solution used for inoculating plants	5	4
	(b) 5 cc. filtrate made up to 50 cc. with distilled water, and solution used for inoculating plants	5	0
4...	(a) 60 gm. $MgSO_4$ added to 100 cc. juice from diseased plants; after standing overnight at room temperature, precipitate filtered out, washed several times with saturated solution of magnesium sulphate; precipitate dispersed by pulping the filter paper in 50 cc. of water. On straining the suspension through cheesecloth a greenish brown solution was obtained which with washings measured 145 cc. This solution used for inoculating plants $2\frac{1}{2}$ days after the salt added	10	9
	(b) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 100	10	9
	(c) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 1000	10	8
	(d) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 10,000	10	3
	(e) Original filtrate from precipitate obtained in experiment 4(a) used for inoculating plants	10	1
5...	(a) 13 gm. Na_2SO_4 (anhydrous) added to 100 cc. of diseased juice; after standing 18 hours at room temperature the precipitate centrifuged out and washed several times with small quantities of a 0.3 saturated solution of sodium sulphate; precipitate then dispersed in 75 cc. of water, and solution used for inoculating plants	10	5
	(b) Supernatant liquid obtained in experiment 5(a) diluted 1 to 100 and used for inoculating plants	10	9
6...	(a) Experiment 5(a) repeated, only variation being that after the Na_2SO_4 was added the solution was held at 32°–33° C. for 18 hours	10	3
	(b) Supernatant liquid obtained from precipitate in experiment 6(a) diluted 1 to 100 and used for inoculating plants	10	9

On attempting to salt out material from the diseased juice at about 0° C., it was found that an immediate precipitate was thrown out only near the saturation point with ammonium sulphate. It was also found that about 34 gm. of ammonium sulphate to 50 cc. of the juice from diseased plants was necessary to produce this precipitate.

Table VI gives the results of some of the experiments on salting out the virus. The plants used to test these solutions were inoculated by scratching three leaves of each plant with a sterilized needle, dropping on the solution used for inoculating, and rubbing it in with a clean cork stopper.

Juice from diseased plants when 25 per cent saturated with ammonium sulphate remained infectious. In the experiments reported in table VI the original supernatant liquid and filtrates were diluted therefore, as indicated, in order to bring the ammonium sulphate concentration below 25 per cent saturation. High concentration of magnesium sulphate is apparently quite effective in salting out the virus. A precipitate forms when juice from diseased plants is 0.3 saturated with sodium sulphate and allowed to stand. As shown in table VI, however, it is doubtful whether this precipitate contains an appreciable proportion of the virus. Work on salting out the virus was soon discontinued, as the precipitates obtained not only contained the excess salt, but also protein and much pigment.

Precipitation of virus of tobacco mosaic with acetone or ethyl alcohol

Since Allard (2) had shown that strong acetone or alcohol rapidly killed the virus, the precipitation by these reagents was first tried out in a cold room in order to reduce as much as possible the injury to the virus. Table VII gives the results obtained.

The preliminary experiments on precipitation of the virus by acetone, as shown in table VII, indicated that a solution of the precipitate, thrown out of juice from diseased plants by two volumes of acetone, is highly infectious. But in order to determine more accurately the proportion of the virus precipitated by adding acetone in the ratio of two volumes to one of the diseased juice, a greater number of plants were inoculated than were employed in the preliminary experiments. The method of Holmes (9) was employed for

TABLE VII

PRECIPITATING THE VIRUS WITH ACETONE

EXPERIMENT NO.	PREPARATION	NO. OF PLANTS INOCULATED	NO. DISEASED
1..	(a) To juice from diseased plants was added an equal volume of acetone in cold; supernatant liquid decanted and precipitate suspended in distilled water; suspension used for inoculating plants	5	5
	(b) Supernatant liquid from above precipitation used to inoculate plants	5	1
2..	(a) To juice from diseased plants were added two volumes of acetone in cold; supernatant liquid decanted and precipitate rinsed with acetone, then absolute ether; precipitate suspended in distilled water and used for inoculating plants	5	5
	(b) Supernatant liquid passed through a hardened paper filter, and filtrate diluted 1 to 3	5	0
3..	(a) Same as in experiment 2(a)	5	5
	(b) Same as in experiment 2(b)	5	0
4..	(a) Same as in experiment 2(a)	5	5
	(b) Same as in experiment 2(b)	5	0
5..	(a) To 25 cc. juice from diseased plants was added 50 cc. acetone in cold; precipitate decanted and precipitate rinsed with acetone, then ether; precipitate suspended in 13.33 per cent acetone and used for inoculating plants	5	5
	(b) Supernatant liquid from precipitate passed through gravity filter and filtrate diluted 1 to 5 (until acetone concentration was about 13.33 per cent)	5	1
6..	(a) Same as in experiment 5(a)	5	5
	(b) Same as in experiment 5(b)	5	0
7..	(a) Same as in experiment 2(a)	5	5
	(b) Same as in experiment 2(b)	5	0
8..	(a) Same as in experiment 2(a)	10	10
	(b) Same as in experiment 2(b)	10	1*
9..	(a) Same as in experiment 2(a)	10	10
	(b) Same as in experiment 2(b)	10	1*
10..	(a) Same as in experiment 2(a)	10	10
	(b) Same as in experiment 2(b)	10	0
11..	(a) Same as in experiment 2(a)	10	10
	(b) Same as in experiment 2(b)	10	3†
12..	(a) Same as in experiment 2(a)	10	10
	(b) Same as in experiment 2(b)	10	2
13..	(a) To juice from diseased plants were added three volumes of acetone in cold; supernatant liquid decanted and precipitate rinsed with ether; precipitate then suspended in distilled water and used for inoculating plants	10	10
	(b) Supernatant liquid passed through hardened filter paper, and filtrate diluted 1 to 3, then used for inoculating plants	10	0
14..	(a) Same as in experiment 13(a)	10	10
	(b) Same as in experiment 13(b)	10	0
15..	(a) Same as in experiment 13(a)	10	10
	(b) Same as in experiment 13(b)	10	0

* One of the blanks was also diseased.

† Three of the blanks were also diseased.

inoculation, whereby only a small amount of virus is introduced by means of fine pins. Table VIII gives the results of these experiments.

From experiments 2–15 of table VII and 3, 4, 7, and 8 of table VIII it is evident that, so far as our present methods indicate, precipitation (under certain favorable conditions) of the virus from juice of diseased plants is, for all practical purposes, complete when two volumes of acetone or absolute alcohol are added to one volume of the juice. The juice obtained from tobacco plants with mosaic disease varies not only in virus content, but also in the content of other solids. It has been observed that when apparently complete precipitation is obtained, the precipitate formed, on adding the acetone or alcohol in the cold, will settle out of solution very quickly and collect in a viscous mass in the bottom of the container. This permits decantation of the supernatant liquid without loss of the precipitate, giving a sharp separation. Under certain conditions, which are not yet entirely clear, a light flocculent precipitate may be obtained which settles slowly. This lengthens the time of contact with the reagent before the supernatant liquid can be decanted. It is also very difficult to decant the supernatant liquid completely from such a precipitate, even after it has settled, without losing some of the precipitate.

Experiments 4 and 7, table VIII, were designed to demonstrate whether loss of activity could be averted by more complete recovery of a precipitate which settled slowly as flocculent material. In this experiment the precipitate was thrown down in a compact mass by centrifuging. The mother liquor was decanted completely without loss of precipitate. Recovery was about complete, in contrast to experiment 2, table VIII, where a light flocculent precipitate was obtained and was not centrifuged. A greater concentration of acetone or alcohol will induce more prompt settling of the precipitate. We prefer, however, to work with the lowest concentration that will produce complete precipitation, as the rate of inactivation must be higher, the higher the acetone or alcohol content. We prefer acetone to alcohol in precipitating the virus, as the precipitate settles much better from acetone solution, and can be redispersed more readily in water.

TABLE VIII

COMPARISON OF AMOUNT OF VIRUS IN ACETONE OR ALCOHOL PRECIPITATE WITH THAT IN ORIGINAL JUICE SAMPLE

EXPERIMENT NO.	PREPARATION	NO. OF PLANTS INOCULATED	NO. DISEASED
1...	(a) 35 cc. juice from diseased plants made up to 100 cc. with C.P. acetone at room temperature, and allowed to stand a few minutes; precipitate obtained on centrifuging dispersed in water, made up to 100 cc., and solution used for inoculating plants	150	54
	(b) 35 cc. juice from diseased plants used in 1(a) diluted to 100 cc	150	74
2...	(a) 100 cc. juice from diseased plants, at about 0° C., added 200 cc. of C.P. acetone at −15° C.; precipitate did not settle, so about 50 cc. more acetone added, then supernatant liquid decanted; precipitate suspended in water and made up to 100 cc.; 50 cc. of this solution diluted to 100 cc. and used for inoculating plants	150	31
	(b) 50 cc. of juice from diseased plants used in 2(a) diluted to 100 cc	150	60
3...	(a) To 100 cc. of juice from diseased plants, at about 0° C., 200 cc. of C.P. acetone at about −15° C. was added; on mixing, precipitate settled at once, giving good separation; supernatant liquid decanted, precipitate suspended in water and made up to 100 cc.; 50 cc. of this solution diluted to 100 cc. and used for inoculating	150	58
	(b) Diseased juice diluted with equal volume of water and used for inoculating	150	51
4...	(a) Two volumes of C.P. acetone at about −15° C. added to juice from diseased plants at about 0° C. (last few cc. of mother liquor removed by centrifuging); precipitate taken up in water and diluted to a volume twice that of juice from which precipitate had been obtained	150	60
	(b) Experiment 4(a) repeated on another sample of same juice	150	64
	(c) Sample of same juice used in 4(a) and 4(b) diluted with an equal volume of water	150	69
5...	(a) 30 cc. of juice from diseased plants made up to 100 cc. with 95 per cent alcohol; precipitate obtained after 30 minutes at room temperature dispersed in water and made up to 200 cc	150	43
	(b) 30 cc. of the untreated juice made up to 200 cc. with distilled water	150	58
6...	(a) 30 cc. of juice from diseased plants made up to 100 cc. with 95 per cent alcohol at room temperature; precipitate thrown down by centrifuging, then dispersed in about 30 cc. of 0.05 N. HCl (pH of dispersion 4.9)	150	45
	(b) Undiluted juice from diseased plants	150	91
7...	(a) 33 cc. of juice from diseased plants at about 0° C. made up to 100 cc. with absolute alcohol at about −15° C.; precipitate thrown down by centrifuging a few minutes at room temperature; precipitate dispersed in 33 cc. of 0.025 N. HCl, then 33 cc. of 0.025 N. NaOH added (resulting pH 5.61)	150	55

TABLE VIII—*Continued*

EXPERIMENT NO.	PREPARATION	NO. OF PLANTS INOCULATED	NO. DISEASED
	(b) Same preparation as 7(a) excepting that precipitate was dispersed in 66 cc. of 0.025 N. HCl (resulting pH 4.8)	150	68
	(c) Same preparation as that of 7(a) excepting that precipitate was dispersed in 66 cc. of distilled water (resulting pH 6.2)	150	52
	(d) Sample of same juice from diseased plants diluted with equal volume of distilled water (resulting pH 5.5)	100	29
8...	(a) 33 cc. of juice from diseased plants made up to 100 cc. with absolute alcohol, then let stand in ice box 30 minutes; precipitate thrown down by centrifuging, washed with ether, then dispersed in 66 cc. of 0.025 N. HCl	150	55
	(b) Same preparation as that of 8(a) excepting that precipitate was dispersed in 66 cc. distilled water	150	76
	(c) Sample of untreated juice from diseased plants diluted with equal volume of distilled water	150	75

HEAT PRECIPITABLE FRACTIONS IN JUICE FROM DISEASED PLANTS

The work on salting out and precipitation with acetone and alcohol made it necessary to determine, if possible, whether simple proteins were connected in any way with the virus. It was soon found that juice from diseased plants contained a fraction that coagulated at about 85° C. When the temperature is raised at the rate of about 1° C. per minute in a water bath, the juice may become turbid near 70° C. The precipitate usually begins to flocculate at 85° C. or sometimes a little below, and the coagulum begins to settle before 90° C. is reached. This coagulum may readily be filtered off. It is very dark in color, and contains considerable nitrogen. The filtrate when heated to boiling gives another precipitate, but this contains only a small amount of nitrogen. Juice from healthy plants also contains these two heat precipitable fractions, but the first fraction may come down at a lower temperature than that from juice of diseased plants.

Table IX gives a comparison of the heat precipitable fractions from juice of healthy and diseased plants. Each precipitate was washed thoroughly by placing in a beaker and boiling with 100 cc. distilled water, filtering, and then repeating the operation once. As

shown from the table, there appears to be no marked difference in the nitrogen content of the two heat precipitable fractions from juice of diseased and healthy plants.

The juice remains infectious after the first fraction is removed by heating, although the virus strength is reduced. Boiling the juice renders it non-infectious.

Partial removal of protein, sulphate, and phosphate

The acetone precipitate formed by adding two volumes of acetone to one volume of the juice from diseased plants contains from 20 to 25 per cent of the dry matter of the juice. This precipitate

TABLE IX

Heat precipitable fractions in juice from diseased and healthy Turkish tobacco plants

Preparation	Precipitate obtained by heating to 89° C.		Precipitate obtained by boiling filtrate, from first precipitate, for 15 minutes	
	Weight (gm.)	Total nitrogen (gm.)	Weight (gm.)	Total nitrogen (gm.)
500 cc. juice from diseased tobacco plants	0.299	0.035	0.114	0.005
500 cc. juice from healthy tobacco plants	0.276	0.033	0.189	0.004

contains the protein fraction coagulating around 85° C., as well as much inorganic sulphate and phosphate. In beginning the work of reducing the dry matter content of the juice, and freeing the virus from more contaminating material, the first point of attack was the protein fraction just mentioned. Fractional precipitation with acetone was unsuccessful in separating the protein from the virus, as 25, 30, 35, 40, and 45 per cent acetone concentration failed to remove either all of the protein or a major part of the virus. It was finally found that most of the protein could be removed with basic lead acetate at a concentration which apparently left the virus unharmed. The solution employed was made by dissolving 200 gm. of Horne's basic lead acetate preparation in 1000 cc. distilled water. This solution is added at the rate of 19 cc. to 500 cc. of the juice from diseased plants. Extensive inoculation experiments have demonstrated that

this concentration of the lead acetate apparently does not reduce the virus concentration; but it does remove most of the protein, some phosphate, and, fortunately, much pigment. After treatment with lead acetate it was found that the acetone precipitate from the cleared juice contained considerable phosphate and sulphate. Barium acetate was then used to remove some sulphate and more phosphate. As much as 10 cc. of a saturated aqueous solution of barium hydroxide with 4 cc. of N. acetic acid have been added to 25 cc. of the juice without apparently destroying a great proportion of the virus. Following the clearing with lead, 20 cc. of a saturated aqueous solution of barium hydroxide with 8 cc. of N. acetic acid were added to 500 cc. of the original juice. Freshly expressed juice from diseased plants has a hydrogen-ion concentration of pH close to 7.0 following lead clearing; consequently, it is necessary to add acid with the barium hydroxide in order to keep the pH on the acid side. The use of 8 cc. of N. acetic acid with 20 cc. of the saturated barium hydroxide solution at 20° to 22° C. will bring the hydrogen-ion concentration of the juice to about pH 6.4 to 6.7.

It has been the practice to allow the juice to stand in a cold room overnight to freeze, following addition of the basic lead acetate solution, and also following the precipitation with an acid solution of barium acetate. In this way solutions showing only slight opalescence are obtained.

A number of preliminary experiments, using sets of ten plants each, and inoculating by the usual method of scratching the leaf surface and then rubbing in the liquid used for inoculating, gave just as many plants diseased from juice that had been treated with the lead and barium solutions as were obtained by inoculating with a sample of untreated juice. To estimate the amount of virus in this fraction the method of HOLMES (9) was employed, and table X gives the results obtained.

PRECIPITATION OF VIRUS FROM JUICE TREATED WITH LEAD ACETATE AND THEN BARIUM ACETATE

After precipitation with basic lead acetate and barium acetate, a flocculent precipitate forms when two volumes of acetone are added to one volume of such treated juice, at about 0° C. This precipitate

settles slowly and never permits complete decantation of the supernatant liquid without loss. After concentrating the treated juice (in vacuo with the bath at 50° C.) to 0.4 of the volume of the original juice taken, a precipitate is obtained when two volumes of acetone are added to one volume of the concentrated solution. This precipitate collects and settles at once, adhering to the bottom of the container. The supernatant liquid may then be decanted immediately

TABLE X

CLEARING JUICE FROM DISEASED TOBACCO PLANTS BY TREATMENT WITH LEAD ACETATE AND BARIUM ACETATE

EXPERIMENT NO.	PREPARATION	NO. OF PLANTS INOCULATED	NO. DISEASED
1..	(a) To 25 cc. juice from diseased plants was added 0.95 cc. of Horne's basic lead acetate solution* and centrifuged; to the supernatant liquid from basic lead acetate precipitation was added 1 cc. of saturated aqueous solution of barium hydroxide with 0.4 cc. N. acetic acid and centrifuged; aliquot from the supernatant liquid, following precipitation with $Ba(OH)_2$, diluted with equal volume of distilled water and solution used for inoculating plants	150	80
	(b) Sample of untreated juice, same as used in preparing 1(a), diluted with equal volume of distilled water and this used for inoculating plants	150	60
2..	(a) Same as in experiment 1(a), except that another sample of juice from diseased plants was used	150	57
	(b) Same as in experiment 1(a), using aliquot of untreated juice from same sample as that used in preparing 2(a)..	150	50

* See text for description.

and drained off completely. More dry matter is eliminated in the latter than in the former case.

Each step in the procedure toward freeing the virus fraction from other solids has been checked numbers of times by the rough method of inoculation previously mentioned, using ten plants in each test. We have tested the activity of the acetone precipitate, from treated juice after concentration, only once by the method of HOLMES (9). Table XI gives the results of this experiment.

The experiment in table XI supports the results obtained with the rougher method. This experiment gives more evidence to the effect that there is little or no loss of virus accompanying precipita-

tion with basic lead acetate, followed by precipitation with barium acetate. For some time we were doubtful as to the effect on the virus

TABLE XI

PRECIPITATING VIRUS WITH ACETONE FOLLOWING CLEARING OF JUICE WITH LEAD ACETATE AND BARIUM ACETATE

PREPARATION		NO. OF PLANTS INOCULATED	NO. DESEASED
19 cc. of basic lead acetate solution added to 500 cc. juice from diseased plants, mixed, placed in cold room overnight; next morning thawed, centrifuged			
Supernatant liquid, decanted, then added 8 cc. of N. acetic acid in 20 cc. saturated aqueous solution of barium hydroxide, mixed, then placed in cold room again overnight; next morning thawed, centrifuged	Precipitate discarded		
Supernatant liquid, decanted into distilling flask and concentrated to little below 200 cc.	Precipitate discarded		
Concentrated, decanted into beaker and made up to 200 cc.; placed in cold room and when freezing 400 cc. of C.P. acetone added (redistilled over soda lime) at about −15° C.; precipitate falls to bottom at once; supernatant liquid decanted completely	Distillate discarded		
Precipitate suspended in about 40 cc. of distilled water; centrifuged	Supernatant liquid discarded		
Supernatant liquid decanted, and made up to 50 cc.	Slight amount of sediment discarded		
1.25 cc. of this solution made up to 25 cc. with distilled water, and solution used for inoculating plants (this volume represents a dilution of original juice with equal volume of water)		150	71
Aliquot from same original juice diluted with equal volume of water, and solution used for inoculating plants		150	68

of concentrating in vacuo below 50° C., but now it seems that the loss here also is small.

Nitrogen, dry matter, and ash content of various fractions

To determine the effect of lead and barium treatment, and precipitation with acetone on the nitrogen, dry matter, and ash content of the fraction containing the virus, the determinations shown on pages 34 and 35 were made, and are representative of the results we have obtained.

The figures in the procedure outlined show that the acetone precipitate obtained, after lead and barium treatment, contains about 10 per cent of the dry matter of the original juice. Present experience indicates that the nitrogen content of juice from diseased plants exceeds that from healthy plants; while the dry matter of juice from healthy plants usually exceeds that from juice of diseased plants. In our system of fractionation, only samples of juice from diseased plants give the immediate precipitate with acetone, following concentration to 0.4 the volume of the original juice sample after lead and barium treatment. No sample of juice from healthy plants has been found to give the characteristic precipitate with acetone at this point in the procedure. A turbidity forms when the two volumes of acetone are added to the concentrated solution, following lead and barium treatment of healthy juice; but an appreciable precipitate settles to the bottom of the beaker only at the end of 10 or 15 minutes.

Preliminary experiments in inoculation, after treatment of the sample with hydrogen sulphide and following a second precipitation with acetone, indicate that the solutions obtained are quite active. We have not, however, made a quantitative study by the method of Holmes at this point in the procedure.

Preliminary experiments indicate that a solution of the second acetone precipitate is inactivated when incubated with trypsin, or more especially by a combination of trypsin and pepsin; pepsin alone seems to have no effect on the virus.

Discussion

The present concepts of the nature of the virus of mosaic disease of tobacco are based, in part, on the following: the resistance to inactivation in 2 per cent phenol solution, 2 per cent creolin solution,

Juice from diseased plants, freshly expressed:

	gm.
Total nitrogen per 500 cc	0.252
Dry matter per 500 cc	12.172
Ash per 500 cc	5.650

500 cc. of this juice plus 19 cc. of Horne's basic lead acetate solution placed in cold room overnight, thawed, centrifuged

Supernatant liquid, decanted, and added 8 cc. of N. acetic acid in 20 cc. of saturated aqueous solution of barium hydroxide; placed in cold room overnight, thawed, centrifuged — Precipitate discarded

Supernatant liquid, decanted into distilling flask and concentrated in vacuo to a little less than 500 cc. — Precipitate discarded

Concentrate, decanted into 500 cc. volumetric flask and made up to the mark (excess lead not removed here) — Distillate discarded

420 cc. concentrated to little less than 140 cc. in vacuo

80 cc. used for analyses:

	gm.
total nitrogen in the 500 cc	0.177
dry matter in the 500 cc	10.365
ash in the 500 cc	4.376

Concentrate, placed in cold and when freezing two volumes of C.P. acetone added at $-15°$ C. — Distillate discarded

Precipitate suspended in 50 cc. distilled water, centrifuged — Supernatant liquid discarded

Clear supernatant liquid, transferred to 200 cc. volumetric flask and made up to volume — Slight residue

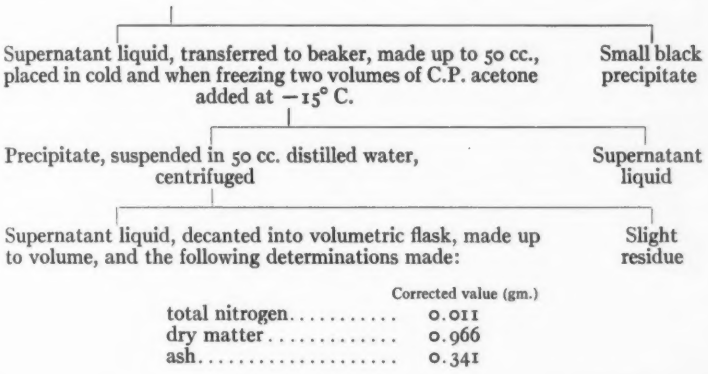
120 cc. $+H_2S$ centrifuged

80 cc. used for analyses:

	Corrected value (gm.)
total nitrogen	0.018
dry matter	1.343
ash	.469

Supernatant liquid, concentrated in volume to less than 50 cc. — Small black precipitate

Concentrate, placed in cold overnight, then thawed, centrifuged — Distillate


Supernatant liquid, transferred to beaker, made up to 50 cc., placed in cold and when freezing two volumes of C.P. acetone added at −15° C.
Small black precipitate
Precipitate, suspended in 50 cc. distilled water, centrifuged
Supernatant liquid
Supernatant liquid, decanted into volumetric flask, made up to volume, and the following determinations made:
Slight residue
Corrected value (gm.)
total nitrogen..... 0.011
dry matter..... 0.966
ash..... 0.341

and 1 per cent phenoco solution (2) is greater than that of vegetative forms of pathogens. The retention of activity in 50 per cent alcohol and 40 per cent acetone, accompanied by a complete loss of activity in higher concentrations (2), indicates less stability toward these reagents than cultures containing spores of *B. subtilis* (6), which after ten days in 99 per cent alcohol or 60 per cent acetone exhibit undiminished viability. Recently MULVANIA (13) has reported that the virus is not completely inactivated when exposed to ultra violet light 30 to 45 minutes; and a faint trace of activity remains after exposure to sunlight for 36 hours. This compares with a period of one to five minutes ultra-violet irradiation and one to two hours' exposure to sunlight required to kill bacteria either in vegetative or spore form. MULVANIA has summarized data to show that the virus of mosaic disease of tobacco is inactivated by a temperature intermediate between the thermal death point of vegetative and spore forms of pathogens.

WOODS (17) was the first to suggest that the virus of the mosaic disease of tobacco was enzymic in nature. FREIBERG (7), after reviewing the evidence available on its precipitation by alcohol, adsorption on talc and inactivation by high concentrations of alcohol, by low concentrations of formaldehyde and at high temperatures, also expressed the view that the active agent was an enzyme.

We have found that when precipitation of the virus is carried

out under favorable conditions, with the proper concentration of safranin, acetone, or ethyl alcohol, the precipitation is almost complete. In each case the precipitate contains practically all of the original activity of the juice, and the virus concentration in the supernatant liquid is no greater than that obtained by diluting a fresh juice sample one thousand-fold. This, together with the fact that the virus is apparently held in an inactive condition in the safranin precipitate and is released when the safranin is removed, makes it probable that the virus which we have investigated reacted as a chemical substance.

Summary

1. An aqueous solution of safranin precipitates the virus of tobacco mosaic from juice of diseased plants. This precipitate brings down practically all of the virus. The virus is apparently held in an inactive condition in the precipitate, but is released when the safranin is removed by means of amyl alcohol.

2. Material, which gives an infectious solution when redissolved in water, has been salted out of infectious juice with ammonium sulphate and also with magnesium sulphate.

3. Two volumes of acetone or alcohol, when added to one volume of juice from diseased plants, at about 0° C., throws down a precipitate which contains practically all of the virus.

4. Juice from Turkish tobacco plants with mosaic disease contains two well defined heat precipitable fractions. One comes down around 85° C. and the other above 90° C. Only the first fraction contains an appreciable amount of nitrogen. The juice remains infectious after removal of the first fraction, although the virus concentration is greatly reduced.

5. Some of the phosphate, sulphate, and most of the protein and pigment may be removed, from juice of diseased plants, with low concentrations of lead acetate and barium acetate without apparently removing or injuring the virus.

6. Juice from diseased plants, after clearing with lead acetate and barium acetate, may be concentrated in vacuo, below 50° C., without apparent injury to the virus.

7. Cleared juice from diseased plants concentrated in vacuo to

0.4 the original volume and brought to about 0° C. gives a precipitate when two volumes of acetone at −15° C. are added. This precipitate contains only about 10 per cent of the solids of the original juice, but apparently contains all of the original virus.

8. The behavior of the virus is in many ways analogous to that of a chemical substance.

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LOCAL LESIONS IN TOBACCO MOSAIC[1]

FRANCIS O. HOLMES

(WITH ELEVEN FIGURES)

Introduction

The literature concerned with the virus diseases of plants repeatedly emphasizes the systemic nature of these infections. MAYER (6), in his original description of tobacco mosaic in 1886, stated that symptoms do not develop on the leaf inoculated, but appear on all of the young developing leaves. Essentially the same account of the course of the disease was given by BEIJERINCK (2) in 1898, by IWANOWSKI (5) in 1903, by ALLARD (1) in 1914, and by others in more recent years.

No detailed descriptions of local lesions developing at the points where tobacco mosaic virus has been introduced have been published. This has probably been due in part to the fact that the local lesions are not conspicuous in commercial tobacco, *Nicotiana tabacum*, which has been used extensively. Another factor which has made the recognition of the local development of the disease more difficult has been the practice of inoculation by scratching and severe wounding. This tends to obscure the primary lesions by producing dead areas mechanically.

A few references in the literature indicate that local lesions have been observed, although their real nature has not been understood. ALLARD (1) referred to *N. langsdorffii* as follows:

> Plants of this species when inoculated through the stalk and petioles seem particularly susceptible to a very destructive and progressive rot, which begins at the point of inoculation and finally kills the plant by slowly involving the surrounding tissues. *Nicotiana viscosum* (*N. glutinosa*) is sometimes killed in exactly the same manner.

FERNOW (3) said of *N. rustica:* "The leaves generally turn yellow and then brown near each point of inoculation." The briefness of

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

these references to the local lesions developing at the site of inoculation with tobacco mosaic virus indicates that these lesions were not recognized as symptoms of the disease.

There is in the literature one account of a plant virus which produces lesions at the point of inoculation. This is the virus of ring-spot of tobacco, which PRIODE (7) describes as forming typical rings of necrotic tissue around needle punctures used to introduce it into tobacco and petunia. These local lesions are exactly like the lesions later produced when the disease becomes systemic; but tobacco mosaic has never been shown to act in this way. Most writers have specifically stated or implied that the inoculated leaf if fully developed never shows symptoms of the infection.

In the present paper the local lesions caused by the introduction of tobacco mosaic virus into the tissues of a number of *Nicotiana* species will be described, and the usefulness of these lesions in measuring the concentration of mosaic virus samples will be shown.

In a former paper (4) the writer has described a method of inoculating plants by means of very small needle punctures. This method is well suited to the demonstration of the occurrence of local lesions. The absence of extensive dead tissue around such needle punctures when transmission does not take place makes the slightest deviation from the normal condition of the leaf tissues conspicuous. The present study was undertaken because evidence was obtained that changes take place at the site of inoculation of tobacco mosaic virus into *N. tabacum*. In this plant it was noticed that pale yellow areas sometimes develop around one or more of the five pin pricks made in each plant. Since in some cases a very dilute virus was being measured, not many plants were expected to take the disease. This fact made the pale yellow areas particularly noticeable, and led to a later examination of the plants which showed them. It was found that every plant which showed such local changes around one or more of the pin pricks developed mottling within a few days. Most of the plants which did not show pale yellow areas near the inoculation punctures remained healthy. This suggested that these yellow areas might represent localized symptoms of a primary infection, since they were consistently followed by the familiar systemic symptoms. Unfortunately the pale yellow areas were very incon-

spicuous and could not be detected in all cases in which systemic symptoms developed.

It seemed possible that some species of *Nicotiana* might show local symptoms more conspicuously and more consistently than *N. tabacum*. A survey of a number of species[2] was therefore made. These were *N. rustica*, *N. trigonophylla*, *N. plumbaginifolia*, *N. longiflora*, *N. tomentosa*, *N. suaveolens*, *N. quadrivalvis*, *N. paniculata*, *N. sylvestris*, *N. sanderae*, *N. glutinosa*, *N. nudicaulis*, *N. langsdorffii*, *N. clevelandii*, *N. acuminata*, *N. glauca*, and *N. multivalvis*. Of these species, five showed pronounced necrotic local lesions instead of pale yellow areas. These species were *N. rustica*, *N. langsdorffii*, *N. acuminata*, *N. sanderae*, and *N. glutinosa*. A description of the local lesions in each of these five species follows.

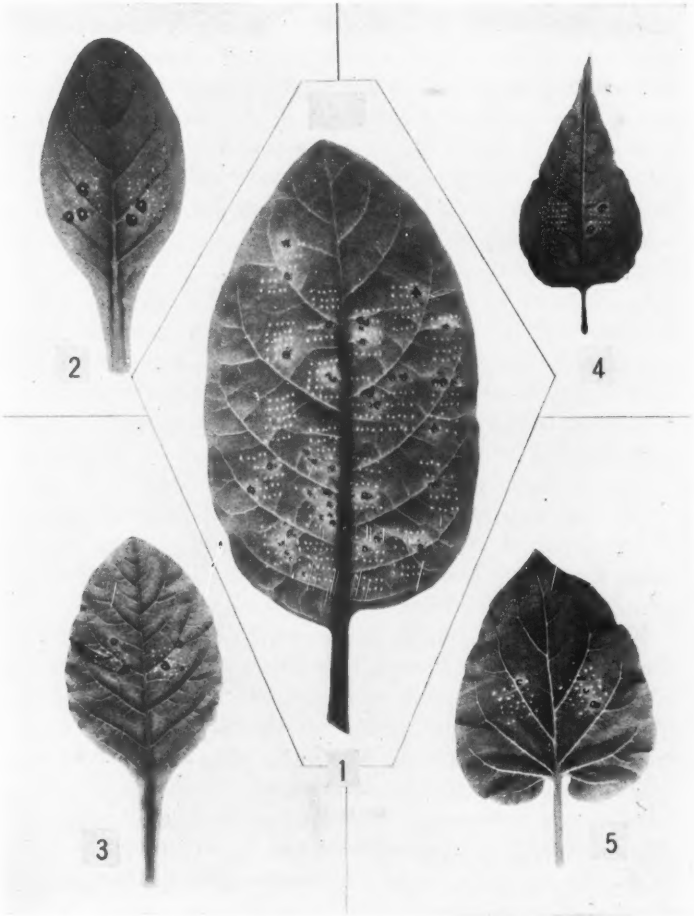
In *N. rustica*, as in all of the species studied, pin pricks which fail to transmit the virus heal perfectly with no macroscopic trace of necrosis; successful transfers, on the other hand, are marked by necrotic spots. These are easily counted eight to ten days after the time of inoculation, and may be distinguished even when many are on the same leaf (fig. 1). These necrotic spots on *N. rustica* are brown in color, circular in some cases, but frequently irregular in outline. They increase slowly in size during the first week after they appear. Later they spread rapidly, especially in young leaves. They may involve a considerable area of the leaf surface before the leaf concerned becomes old and drops off.

The lesions on *N. langsdorffii* (fig. 2) and *N. sanderae* (fig. 3) are more conspicuous than those on *N. rustica*, because they are blackish with concentric rings of dead tissue. They grow larger day by day after their first appearance, and may become very extensive. Sometimes veins and even the stem of the plant are involved in the slowly spreading necrosis of the tissues.

In *N. acuminata* (fig. 4) the lesions are irregular in outline and brown in color. They appear one or two weeks after the introduction of the virus, and increase in size rather more slowly than is the case in the other species.

[2] Thanks are due to Dr. S. A. WINGARD of the Virginia Agricultural Experiment Station, Dr. R. E. CLAUSEN of the University of California, and Dr. E. M. EAST of the Bussey Institution, Forest Hills, Massachusetts, for their kindness in supplying seeds of these species.

N. glutinosa exhibits a very different response (fig. 5). The lesions begin to be noticeable on the second or third day and are all completely formed by the fifth day, when in other species the



FIGS. 1–5.—Local necrotic lesions in: (1) *N. rustica*, (2) *N. langsdorffii*, (3) *N. sanderae*, (4) *N. acuminata*, (5) *N. glutinosa*. Lesions in *N. rustica* and *N. acuminata* are brown, in *N. langsdorffii* and *N. sanderae* almost black, and in *N. glutinosa* pale brown surrounded by rings of darker brown.

first indications of breakdown appear. These rapidly developing necrotic lesions appear first as tiny glistening dark spots. The centers soon dry down and develop a light brown color. Around them darker brown rings form concentrically. This gives the lesions an easily recognized appearance not to be confused with other dead spots in the leaves.

In order to discover whether these necrotic local lesions consistently appear whenever a successful transfer of the mosaic virus is accomplished, a test with small *N. rustica* plants was arranged. In a number of experiments each plant was inoculated with a single pin prick in a young leaf. The inoculation was carried out with a no. oo black enamel insect pin previously wet with juice from a mosaic leaf of commercial tobacco. Altogether 582 plants were thus inoculated. Of these, 136 took the disease, as shown by the development of the severe systemic symptoms characteristic for this species. Every one of these plants showed a local necrotic lesion at the site of inoculation. These lesions were similar to those shown in fig. 1, except that there was but one on each plant. The fact that every plant which took the disease showed a local lesion is evidence that the local lesion is a definite symptom of the mosaic. Six plants in the series showed local lesions without a later development of the systemic disease. Apparently the virus was not able to spread in these individual cases. Their number is so small, however, that the diagnosis of transfer of the disease is not much changed if local lesions are taken as evidence of the transfer without waiting to examine the later systemic symptoms. Later experiments, in which more than one pin prick was used for each plant, have substantiated these conclusions.

Further evidence that the successful transfer of virus can be detected by examination of the local necrotic lesions is afforded by the fact that concentrated virus samples cause the development of large numbers of these local lesions when they are used as inoculum, whereas known dilutions of the virus samples in water cause the development of smaller numbers of the lesions. Graphs showing the accuracy with which the numbers of lesions are dependent upon the known concentrations of virus samples will be presented later in this paper, when the use of the local lesions in measuring virus concentrations is discussed.

No bacteria or other visible microorganisms have been found upon microscopical examination of the local lesions. Fluids from plants not affected with mosaic do not produce the lesions when used as inoculum. Yet it appeared that formal experiments ought to be carried out with virus suspensions free of bacteria to show that the production of the lesions was not due to the introduction of foreign organisms. Three samples of virus were therefore prepared by adding large numbers of a small bacterium, *Aplanobacter michiganensis*, to virus previously diluted with seven volumes of water. These three samples were then passed through Berkefeld W filter candles. The bacteria were entirely removed from the mixture, as was shown by plating from the filtrates. The virus passed through the filter with no observable decrease in its concentration. The lesions developing on *N. glutinosa* plants inoculated with these sterile filtrates were identical in appearance with those produced by the use of unfiltered samples of virus.

In further support of the view that the virus of mosaic itself is responsible for the production of these local lesions, it may be stated that exactly similar lesions have been produced by the use of a virus sample which had been frozen solid for three years, and by the use of several field samples of virus collected at widely separated points. It is improbable that any second virus or any other organism would be so closely associated with tobacco mosaic virus as to be present in each of these cases.

In order to prove beyond doubt that the lesions are not caused by any foreign organism, they should be produced in a sterile plant by the use of virus freed from bacteria. But the fact that the test plants are grown in sterilized soil from seeds seems sufficient protection against the presence of any organisms on their surface, especially as the individual plants are invariably capable of showing the lesions.

Use of local lesions in measuring virus concentrations

It has long been the custom to use the appearance of the systemic disease in *N. tabacum* plants as an indicator of the successful transfer of virus from mosaic plants or extracts from them. Several methods of this kind are in use for the more or less accurate measurement of

the relative concentrations of mosaic virus in different samples. In such work a whole plant is necessary for each successful transfer. As the accuracy of the measurement depends largely upon the number of successful transfers, the numbers of plants required usually prove a limiting factor in the number of measurements which can be made. Obviously it would be a great advantage if many successful transfers could be distinguished on a single plant or even on a single leaf. This advantage is given by the use of the local necrotic lesions here described.

It has been found that the number of successful transmissions of virus can be learned by counting the local necrotic spots at the points of inoculation in such species as *N. rustica* and *N. glutinosa* as well as by using whole plants as indicators. Moreover, since the lesions appear locally before systemic symptoms appear, economy of time is secured.

Since *N. rustica* offers the advantage of very large leaves, a method of using it for measuring virus concentrations will first be described. Dilution curves and a discussion of the accuracy attainable will be given. *N. glutinosa* has proved of great value, because on it necrotic lesions, which can readily be counted, appear very soon after inoculation. The disease may be transmitted by wiping the leaf surface gently with a cloth saturated in virus extract.

In *N. rustica* lesions are most readily counted when produced by pin prick punctures. A set of five insect pins held in a temporary handle has usually been used by the writer for introducing the virus. The pins are alternately dipped in a sample of virus and used to puncture the test plant leaf. In this way large numbers of punctures are rapidly made in a series of leaves. Usually each leaf will accommodate 250 or 500 punctures. The number of necrotic lesions developing is small in comparison with the number of punctures, but is sufficient to allow a fairly accurate reading of virus strength to be made with a few plants. In fig. 1 a leaf inoculated as described is shown. It is necessary to make inoculations of samples on opposite sides of the midvein of the same leaf or to use a large number of leaves for a single test. This is because leaves of different ages have been found to differ somewhat in susceptibility. In general the younger leaves are the more susceptible.

A curve showing the effect of dilution over a considerable range of virus concentrations is presented in fig. 6. The graph is based upon two series of experiments, one with a mosaic virus of usual

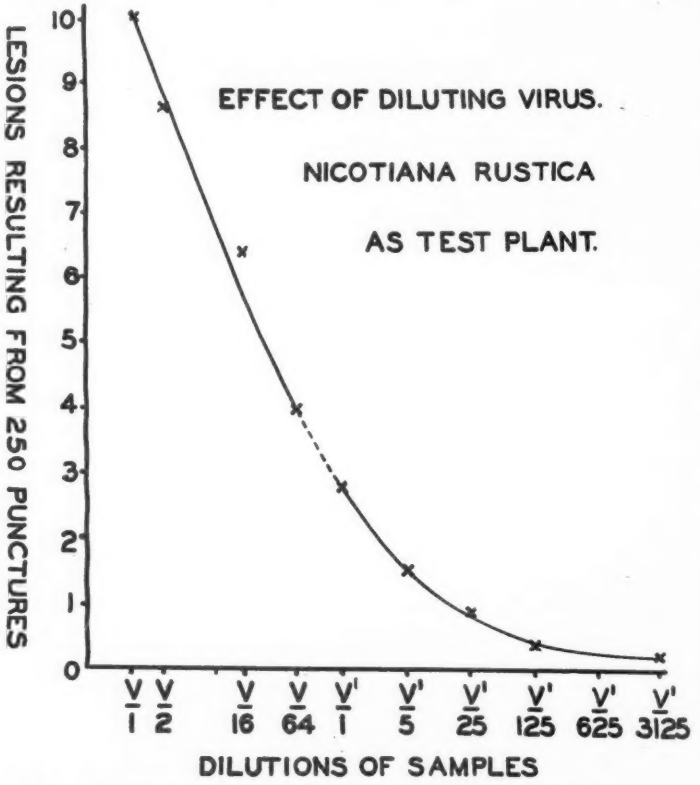


FIG. 6.—Effect of diluting virus when *N. rustica* is used as test plant. Two samples of virus, V and V′, used in obtaining the data represented. Virus V was more concentrated than virus V′, which was exceptionally weak. The lesions resulting from 38,000 pin prick punctures are represented by the nine points determining the curve.

strength and one with a weak sample. Thirty-eight thousand punctures were made to obtain the transfers represented by the figure. It will be observed that in the region in which four to ten lesions were obtained in each set of 250 punctures, the line appears nearly

straight as drawn to a semilogarithmic scale. This part of the curve is similar to that known from earlier work with commercial tobacco. It is interesting to note the direction of the line when more dilute virus is used. With *N. rustica* it has been possible to study the lower range, because greater numbers of measurements can be made by the use of local lesions than by the use of the systemic disease as an indicator of successful transmission. The upper range is the portion most frequently dealt with, and the range in which the greatest accuracy can be obtained.

Nicotiana glutinosa as test plant

The characteristics of *N. glutinosa* make it a very useful test plant for measuring virus concentrations. It has a low virus content when diseased, which gives it an advantage over *N. tabacum*, in that contaminations do not readily occur when the plants are handled. The rapidity of development of the local lesions makes it possible to have preliminary results of measurements on the second or third day after inoculation, according to the season. On the fourth or fifth day final results may be noted and the plants discarded. Large numbers of lesions can be distinguished on individual leaves; thus a high degree of accuracy may be obtained in comparing virus concentrations. The use of pin punctures is unnecessarily slow in the use of this species as a test plant. In its stead a much more rapid method of inoculation may be used, allowing tobacco mosaic virus to be measured as readily and as rapidly as bacteria are counted by plating methods.

The procedure is as follows. *N. glutinosa* plants are grown in 4-inch clay pots until flower buds begin to appear. At this stage at least five leaves on each plant are of good size. These five leaves are used for the inoculation; and for convenience in manipulation all the remaining leaves of the plant, as well as the growing point, are pinched off. This leaves a sturdy stem supporting five large leaves. Virus from any source to be tested is now taken up on a small piece of white cheesecloth and rubbed once firmly but gently over the entire upper surface of the five leaves. A full stream of tap water is used to wash away excess virus at once after this inoculation. After a little practice the operations become quite uniform. The results

justify the belief that approximate uniformity of inoculation can thus be obtained. Undiluted tobacco mosaic extracts result in the production of about 300–600 lesions on each test plant when applied in this way. If they are diluted with water, the decrease in the number of lesions is at first very rapid, later more gradual. A wide range of concentrations can be studied accurately.

As an illustration of the effectiveness of this method of inoculation, a series of leaves showing the decrease in the number of lesions appearing with decreasing concentrations of virus in the inoculum is shown in fig. 7. A dilution curve showing the effect more accurately is shown in fig. 8. Fig. 9 shows the lower range of this curve more clearly. A curve showing the probable errors of counts of lesions on single test plants is shown in fig. 10. By reference to these curves it will be observed that a very slight dilution, as by a single volume of water, can be detected with satisfactory accuracy by the use of a small number of test plants. In most preliminary measurements a single test plant is sufficient to give an excellent idea of the strength of the virus sample in hand. Even the most important measurements are usually made accurately enough if sixteen test plants are used. Fig. 11 shows two test plants, one five days after inoculation, the other one day after inoculation.

FIG. 7.—Five leaves from plants of *N. glutinosa* used to measure the effect of dilutions (1:1, 1:3.16, 1:10, 1:100, 1:1000). Numbers of lesions do not correspond to the more exact averages shown in dilution graph in fig. 8, but decrease in lesions with serial dilution is plainly shown.

The value of this method of measuring the concentration of tobacco mosaic extracts can best be appreciated when it is compared with the old method for estimating the strength of a sample by

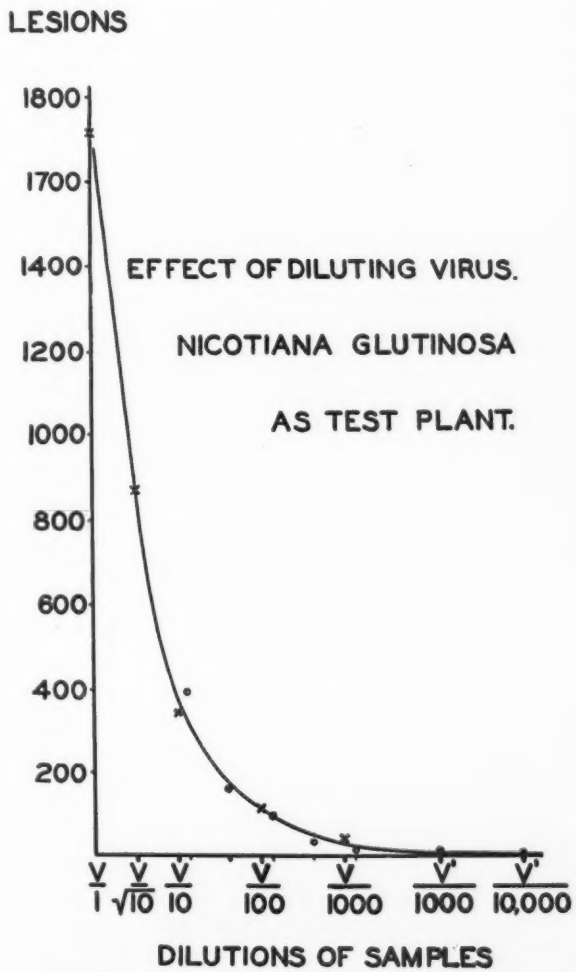


FIG. 8.—Effect of diluting virus sample, *N. glutinosa* as test plant. Counts of lesions in every case represent average number of lesions appearing for each test plant, when five leaves of each plant had been wiped with the virus sample in question. Accuracy attained may be noted by closeness of determined points to the smooth curve. Nine plants used for each determination; probable error of each point as determined is therefore one-third the error shown in fig. 10 for single observations of similar numbers of lesions.

inoculating potted plants of *N. tabacum*. Ten plants of this species were frequently used in a single test. On the average five successful inoculations and five failures would be observed on this number of test plants. On an equal number of *N. glutinosa* test plants in a

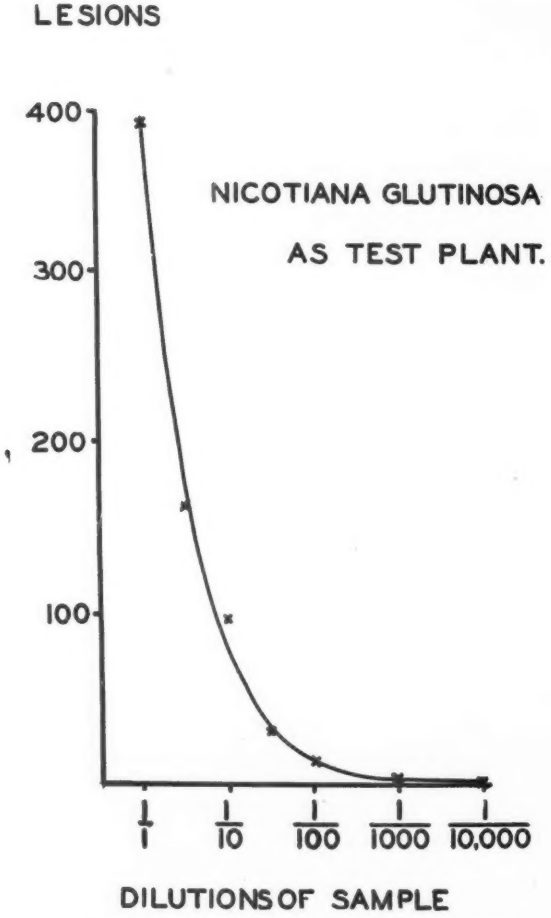


FIG. 9.—More dilute range of curve shown in fig. 8, drawn to such a scale that readings may be made accurately (see fig. 10 for probable errors of single observations in this range of measurements).

typical case 5000 successful inoculations would be observed because of the localized nature of the lesions counted. The accuracy of both

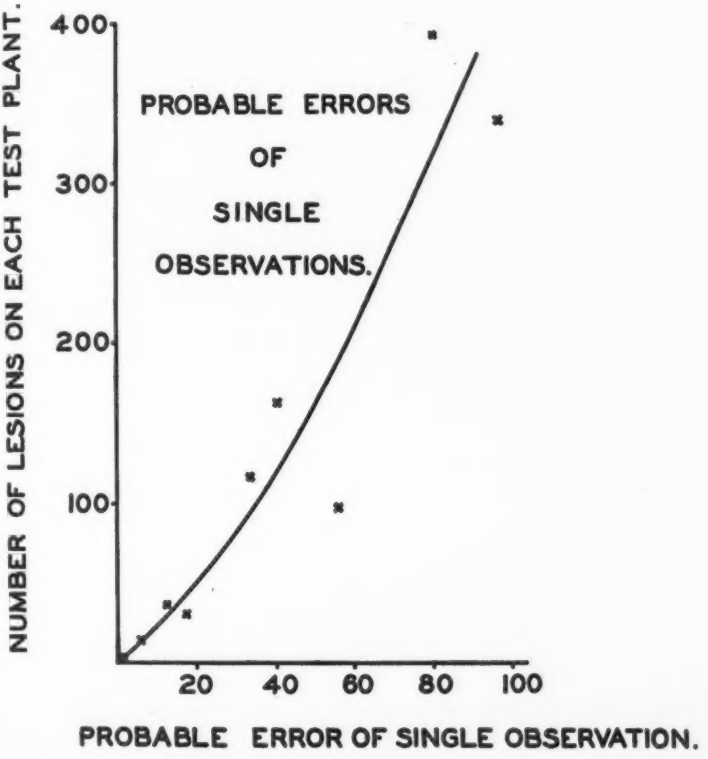


FIG. 10.—Probable errors of single observations for test plants having numbers of lesions between 0 and 400. The error in determining these probable errors is naturally large, and information was insufficient to extend curve beyond 400. At 1714, however, the probable error of a single observation has been found to be 414.

methods depends largely on the number of successful inoculations counted. It is estimated that in the average case for the same accuracy one test plant of *N. glutinosa* used as described serves the purpose for which at least several hundred *N. tabacum* plants are required with the customary methods of using that species.

N. glutinosa differs from the other species of *Nicotiana* in two ways. It produces very little virus when successfully inoculated, and symptoms appear on the upper parts of the plant to a very limited extent. Measurements of the amounts of virus produced by this plant have shown that the concentration present does not exceed the strength of an ordinary sample of commercial tobacco mosaic virus diluted with water to one-five hundredths of its original strength. The systemic course of the disease is not like that of most

FIG. 11.—Test plant of *N. glutinosa* (at left) as used to determine virus concentrations, inoculated five days before it was photographed. Similar plant (at right) inoculated one day before it was photographed. Lesions had not yet appeared on this recently inoculated plant.

species. Generally the growing tip soon shows the same type of symptoms which have occurred where the virus was introduced. The writer has never seen a case in which the developing leaves of *N. glutinosa* were affected with necrotic spots. The local lesions enlarge day by day, laying down ring after ring of dead tissue. After a week or so new secondary spots are sometimes formed on the peripheries of the extended primary lesions. Later lesions may appear on leaves younger than those inoculated, but they do not affect the developing leaves. Sometimes veins and portions of the stem are killed by the extending lesions. The green portions of the leaves between the primary lesions are not suitable sources of virus. No successful trans-

fers have been secured when juice from them has been used as inoculum. The virus seems to be confined to the visible lesions.

N. glutinosa, like *N. rustica*, shows a gradient of susceptibility when successively older leaves are considered. The young leaves tend to produce more local lesions when inoculated with the same source of virus as neighboring older leaves. This condition is partially remedied by removing the growing point of the plant, as is done in using the plant for measurements. In two series of plants, one with the growing tops attached, the other with the tops removed, this condition of affairs was demonstrated. In the first series, in which the growing points remained attached, the totals from top leaf to lowest leaf were 6066, 5117, 4292, 3222, and 3198. The gradient is very marked. In the second series, in which the growing points were removed, the totals were 4795, 4778, 4227, 4320, and 4343. The gradient is much less. The practice of removing the tops insures less error in comparing leaves of different age, since the chance choice of an older or a younger leaf in any plant will introduce less variation when the difference in the susceptibility is decreased.

Discussion

The recognition of symptoms which appear at the site of inoculation opens a new field of investigation in connection with tobacco mosaic. The first development of the disease seems to be very strictly localized. It is only the later course of the infection which is marked by changes in appearance of the developing leaves. The species of *Nicotiana* differ markedly in the conspicuousness of their local symptoms. In some cases necrotic spots are formed, in others a pale yellow area marks the point of entrance and early development of the virus.

Most of what is known regarding virus diseases of plants has been learned from inoculation studies. The behavior of the virus under experimental conditions, such as filtration, purification by chemical processes, and contact with disinfectants has always been judged by its reinoculation into living plants. Such tests have been difficult to carry on with a high degree of accuracy, because of the large numbers of plants required for each determination. The use of local necrotic lesions makes it possible to recognize very large num-

bers of successful transmissions on single plants. This reduces the amount of labor in many experiments. A degree of accuracy never before possible can be attained in this way with the use of moderate numbers of plants. *N. glutinosa* lends itself particularly to use in measuring virus concentrations, since the necrotic lesions in this plant develop in from two to five days. These lesions are easily counted and their relative numbers show variations in the concentrations of samples with great clearness.

When very small numbers of lesions develop on *N. glutinosa* because of the use of highly diluted samples of virus, there occur many cases in which a plant shows a single local lesion. Under such circumstances it seems possible that the infection has resulted from the entrance of a single virus particle. Transfers from this type of plant may be assumed to develop virus produced from this single particle. The process of isolation may be repeated if desired. Possibly a pure line of the causal agent is thus secured. Such single particle strains have not yet been carefully studied; their isolation and use may be expected to furnish material for future work. The use of *Nicotiana* species which show local necrotic lesions is as helpful in the study of tobacco mosaic virus as KOCH'S plate method is in the study of bacterial cultures.

Summary

1. Five *Nicotiana* species were found to develop necrotic lesions wherever virus of the common field type of tobacco mosaic successfully entered leaf tissues. These species were *N. rustica*, *N. langsdorffii*, *N. sanderae*, *N. acuminata*, and *N. glutinosa*.

2. The local lesions developing in *N. rustica* can be used to measure accurately the potency of tobacco mosaic virus.

3. The local lesions in *N. glutinosa* are exceptional in the rapidity of their development. They sometimes begin to appear thirty hours after inoculation. In four or five days they are well developed. Large numbers of them may be distinguished on a single plant. This allows comparisons to be made between virus samples, since the number of lesions developing depends on the virus concentration of the inoculum.

4. A standardized method for using *N. glutinosa* as a test plant for measuring the concentration of mosaic virus gives as rapid and as

accurate results as the determination of bacterial numbers by plating methods.

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INOCULATING METHODS IN TOBACCO MOSAIC STUDIES[1]

FRANCIS O. HOLMES

(WITH FOUR FIGURES)

The virus of tobacco and tomato mosaic disease has usually been transferred from plant to plant in mosaic studies by scratching or pricking leaves, and wetting the injured areas with extracts from mosaic plants. As a rule the virus is gently rubbed into the wounds. This process successfully transfers the infection and has been accepted widely. Occasional failures to secure the expected results suggested the advisability of making an analysis of inoculation methods. The purpose of the present study was to ascertain which methods of introducing virus were most effective.

At the beginning of the work, the problem in hand was the simple question of the proper depth of scratch to make in the surface of a leaf before wetting the area with virus. The method used was that described in the preceding paper.[2] *Nicotiana rustica* plants were inoculated in the ways which were to be tested, and the numbers of lesions resulting were used as indications of the numbers of successful transfers. This process soon showed that different types of wounds were very different in effectiveness.

To determine whether shallow or deep scratches were the more effective in aiding the virus to enter the tissues, the following experiment was performed. Several series of scratches, some shallow and some deep, were made on individual leaves of the test plants. Virus was applied at once by wetting a small piece of cheesecloth and lightly wiping the surface of the scratched leaf. It was expected at the time that the virus would enter some types of wound more readily than others, and that numerous necrotic lesions would cluster around the scratch of most favorable depth. If this had been the

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

[2] HOLMES, F. O., Local lesions in tobacco mosaic. BOT. GAZ. **87**: 39–55, 1929.

case, it would have been clear whether broken surface cells or deeper layers were the more receptive. When the lesions appeared, however, it was found that very few of them were on or near the scratches. They were well distributed over the general surface of the leaf between and around the scratches. A typical leaf from such an experiment is shown in fig. 1. Apparently the scratching had been an unimportant part of the inoculation method. The light rub-

FIG. 1.—Leaf of *Nicotiana rustica* on which 20 scratches were made with needles, and virus was rubbed over the scratches and whole surface of the leaf. Resulting lesions were photographed when small, and appear as tiny dark spots, very few of which are on or near the scratches. On original leaf at time of photographing the lesions were brown, the scratches green like the general leaf surface.

bing must have produced most of the wounds necessary for the entrance of the virus.

To secure further evidence on this point another experiment was carried out. The scratching and rubbing operations were separated. On one side of the midvein of a leaf of a healthy test plant ten scratches were made. On these an extract from mosaic plants was dropped and spread with care to avoid injuring the surface near the wounded area. In the same way 200 scratches were made on twenty leaves. On the other side of the midveins the same virus was rubbed lightly into small areas of the leaves. No scratches were made here, nor was any distinct injury to be seen after the application of the virus. On a given leaf of *Nicotiana rustica* the tissues

on one side of the midvein of a leaf are precisely as susceptible as the tissues on the other side of the midvein. If the two methods of inoculation were equally effective, equal numbers of local lesions would be expected on each side of the leaves treated. When the lesions appeared, however, it was found that they were numerous on the side of each leaf into which virus had been rubbed; but they were rare or absent on the side which had been scratched and then wet with a liberal amount of the mosaic extract. On the 200 scratches only four lesions developed. Two leaves from a series of such experiments are shown in fig. 2.

A similar test was carried out to discover whether scratching through a drop of mosaic extract was effective. Twenty-five separate leaves were scratched, ten times each, through drops of the fluid. On these leaves the same virus was rubbed into similar small areas. Great numbers of lesions appeared from the rubbing, but only 13 lesions were found on the 250 needle scratches. Scratching through mosaic extract, therefore, appears slightly more effective than dropping virus on scratches, but neither method proved to be efficient as compared with light rubbing. These experiments make it seem probable that when scratching and rubbing methods of inoculation are used, the scratches are relatively ineffective, and the rubbing constitutes the effective portion of the process.

It was not possible to obtain so clear a demonstration of what happened when similar experiments were performed with commercial tobacco, because of the difficulty in detecting the spot through which infection took place. Nevertheless a test was made to see whether the response was similar. Forty-four plants of Turkish tobacco were inoculated by making ten scratches on each and immediately wetting these wounds with mosaic extract. Forty-four additional plants of the same lot were inoculated with the same mosaic extract by the simple process of rubbing lightly over a small area with a piece of cheesecloth, wetted in the same source of virus. When the plants began to show symptoms, it was found that only eight successful inoculations in forty-four trials had occurred in the group inoculated by scratching and wetting the scratches with virus. On the other hand, forty successful inoculations had resulted from the forty-four trials in the group subjected to light rubbing with

cheesecloth wet with mosaic extract. This experiment shows that *Nicotiana tabacum* behaves like *N. rustica*, in that it is not readily

FIG. 2.—Two leaves of *Nicotiana rustica* typical of a large series in which scratches were made on one-half of each leaf and immediately wet with virus extract from a dropper; on remaining half a small area was rubbed gently with cheesecloth saturated with the same virus used to wet the scratches. Rubbing proved more effective than scratching as an inoculating method.

inoculated through scratches wetted with virus, but is more easily inoculated by light rubbing.

It seemed desirable to know whether wounds made by rubbing would be more effective when made in the presence of the virus, or whether they could be made and subsequently inoculated with equally good results. To test this a comparison was obtained in

the following way. A piece of dry cheesecloth was used to rub one-half of a leaf. Mosaic juice was then dropped at once upon this surface. The same kind of cloth wetted with the same source of virus was then used to rub the opposite half of the leaf. Lesions developed in abundance on leaf areas rubbed with cloth wet with virus, but areas rubbed first with a dry cloth and then wetted with virus developed very few necrotic spots. This is illustrated by the leaf shown in fig. 3. It was found that if a water-soaked cloth was used to break the hairs instead of a dry cloth, the result of the experiment was the

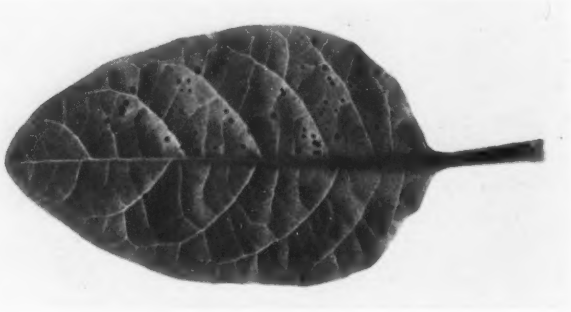

FIG. 3.—Leaf of *Nicotiana rustica* on one-half of which virus was dropped after rubbing with cheesecloth had broken the hair cells (one lesion appears); on the other half the rubbing was with cheesecloth saturated with sample of juice used on the other half. Rubbing is effective as an inoculation method only in presence of the virus.

same. The virus did not enter the wounds made before it was applied, but required wounds made in its presence.

If virus cannot readily enter wounds a few seconds after they are made, it must be that the actual entrance is completed almost instantaneously; otherwise wounds would quickly become useless although made in its presence. If the virus enters instantaneously, the practice of renewing mosaic extract above wounds and keeping it there for long periods to allow extended opportunity for entrance may be useless. To test whether it is or not the following experiment was performed. On one side of a leaf the hairs were broken with a cloth wet with water, and the area then flooded with fresh extract from a mosaic plant. On the other half of the leaf the same

kind of cloth was used, after wetting it with the same juice, to rub lightly the remaining leaf surface. As soon as the process was complete a full stream of water was used to wash the virus from this second half of the leaf, but virus was renewed over the water-rubbed portion so as to keep it wet for several minutes. Thus the first portion of the leaf was apparently favored by long contact with the virus; yet an overwhelming majority of the lesions appeared on the portion rubbed for a moment with virus and then washed clean with a stream of water. Many repetitions of this process showed the same result.

This experiment supplied additional evidence that wounds made immediately before the application of virus were ineffective. It also indicated that when virus was applied directly, it was taken up so quickly that the excess might be washed away at once without making the inoculation ineffective.

To test the possibility that the washing away of the excess virus might decrease the count to a slight extent, a quantitative experiment was performed. A group of plants were inoculated by rubbing the whole leaf surfaces with mosaic juice. As soon as each leaf was treated, one-half was washed with a stream of water. When the lesions appeared they were carefully counted for the washed and unwashed halves of the leaves. Normally the two halves of the leaves would show approximately equal counts and there would be a chance variation from side to side, but under these two treatments there was a definite response in favor of the washed portions. On the washed halves there were 1221 lesions, and on the unwashed halves 964. Nine of the twelve leaves counted showed excess lesions on the washed portions. The exceptions were nearly equal counts on three leaves. It was further noted that on eleven of these twelve leaves the lesions were slightly earlier in appearing on the washed portion. This observation lends further support to the view that the washing favored the inoculation and did not hinder it. Perhaps the water hastened the recovery of some cells by washing away the excess of toxic materials.

These experiments give some insight into what happens when virus is applied to susceptible plants. Small wounds, imperceptible to the unaided eye, take up virus instantly. Wounds made before

virus is applied are nearly or quite useless. Excess of virus may be removed at once with a stream of water without decreasing the number of effective invasions of the plant by the virus.

FIG. 4.—Two leaves of *Nicotiana rustica*, each of which was rubbed over its whole upper surface with cheesecloth saturated with the same sample of virus (preserved in 50 per cent glycerin). Mixture was allowed to stand on surface of leaf at right. Injury from presence of glycerin appears on edges of leaf and few lesions are in evidence. Leaf at left was washed with full stream of water at once after treatment. No injury noticeable in such cases, and abundance of lesions develop.

Practical use of this information can be made. It is often desired to treat virus with chemicals harmful to plant tissues. The virus may survive the treatment, but tests of the resulting mixture may be difficult because of the killing of the leaves by the chemicals

present. If the mixture is applied by rubbing and is then washed off with water to remove the excess, successful inoculations may be obtained.

Fig. 4 shows at the right a leaf rubbed with virus in 50 per cent glycerin. The presence of the glycerin soon caused drying of portions of the leaf and the death of certain areas. A similar leaf treated with the same glycerinated virus is shown at the left. It was washed with water at once after the inoculation. The lesions developed very much more freely in this second case, and the leaf itself showed no injury from its brief contact with the chemical. It seems probable that immediate washing of inoculated leaves when any foreign materials are applied with the virus will allow more successful transfers to be made.

Summary

1. The most effective way of transferring mosaic virus to tobacco plants has been found to be gentle rubbing over a large leaf surface with a cloth soaked in extract from mosaic plants. Scratches are much less effective, even when made in the presence of the virus.

2. The virus of tobacco mosaic does not readily enter wounds made in the leaves of healthy tobacco plants if these are made before its application.

3. Entrance seems to be instantaneous upon the production of a suitable wound in the presence of the virus. Immediate removal of the excess of mosaic extract by washing does not decrease the total number of infections, but in some cases actually increases it.

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MICROCHEMICAL STUDIES OF ROOTING AND NON-ROOTING ROSE CUTTINGS[1]

MARGERY C. CARLSON

(WITH SIX FIGURES)

Introduction

Cuttings of overwintered canes of Dorothy Perkins rose produce shoots which give rise to roots when placed in humid air or in a moist medium (fig. 3). American pillar rose, treated in the same way, produces shoots but no roots, as shown by ZIMMERMAN (14). It seemed that a comparison of the anatomy and composition of such closely related plants with so marked a difference in response might give some idea of the factors influencing their rooting behavior.

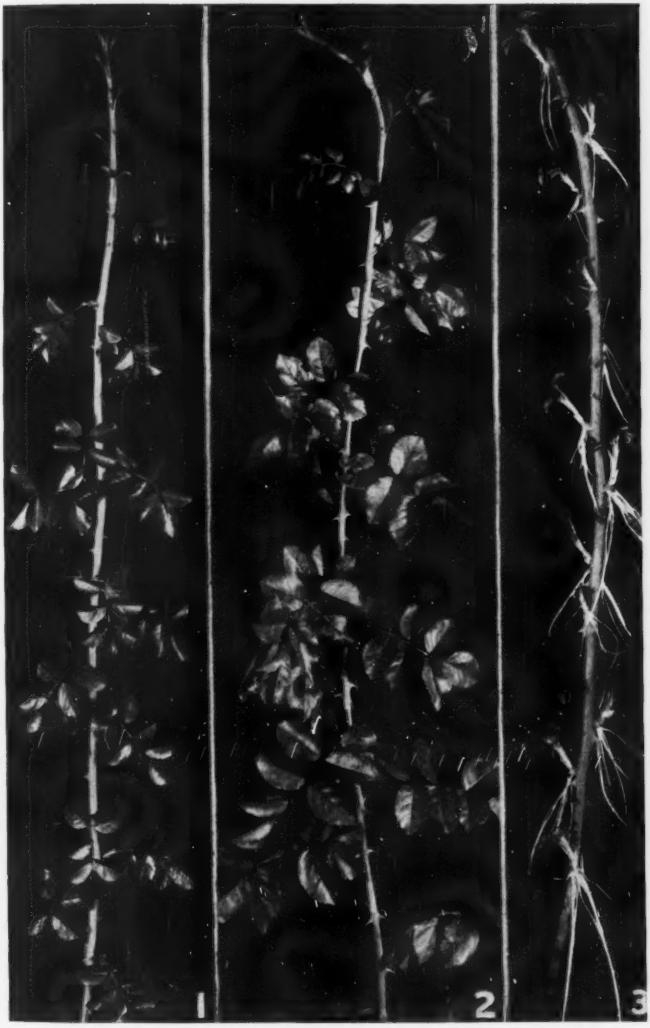
Dorothy Perkins and American pillar are hybrids with a common parent. Dorothy Perkins results from a cross of *Rosa wichuraiana* × Mme. Gabriel Luizet, and American pillar from a cross of *R. wichuraiana* × *R. setigera* (1). They are climbing roses of the *multiflora* type, producing long, unbranched canes one season and flowers the second season. Figs. 1 and 2 show the vegetative canes of each rose. Dorothy Perkins has longer and more flexible canes, smaller leaves, and smaller thorns than American pillar.

Material and methods

Canes were cut into pieces 10–15 inches long, and placed with their basal ends in water in saturated atmosphere, either in a large humidity case in the greenhouse, or under bell jars in the laboratory. The humidity case was aerated only by the frequent opening of the door, but air was drawn through the bell jars by means of a filter pump.

Microchemical examinations of transverse and longitudinal sections of nodes and internodes were made at the time of collection,

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.



FIGS. 1–3.—Figs. 1, 2, vegetative canes of Dorothy Perkins (left) and American pillar (right); fig. 3, cutting of Dorothy Perkins, showing young branches and roots produced in saturated atmosphere.

and at intervals of two to four days throughout the experiments. Six series of examinations were made as follows:

SERIES NUMBER	DATE STARTED	DURATION OF EXPERIMENT (DAYS)
1	March 15, 1926	31
2	March 24, 1926	28
3	April 7, 1926	16
4	April 22, 1926	16
5	March 6, 1927	18
6	April 6, 1927	19

The microchemical tests described by ECKERSON (2) were used.

STARCH.—Iodine solution in potassium iodide.

FRUCTOSE.—(1) Copper tartrate and 20 per cent sodium hydroxide, cold; (2) phenylhydrazine-hydrochloride and sodium acetate, 12 hours at room temperature.

GLUCOSE.—(1) Copper tartrate and 20 per cent sodium hydroxide, heated 1–2 minutes at 40° C.; (2) phenylhydrazine-hydrochloride and sodium acetate, heated 24–48 hours at 40° C.

OTHER REDUCING SUBSTANCES.—Copper tartrate and 20 per cent sodium hydroxide, heated 15–20 minutes at 40° C.

PROTEIN.—(1) Iodine solution in potassium iodide; (2) Biuret reaction: 5 per cent copper sulphate and concentrated potassium hydroxide.

ASPARAGIN.—Absolute alcohol on sections and identification of crystals which precipitate out.

NITRATES.—Diphenylamine in 75 per cent sulphuric acid.

CALCIUM.—(1) Five per cent sulphuric acid; (2) identification of calcium oxalate crystals.

POTASSIUM.—Platinum chloride.

MAGNESIUM.—Ammonium chloride, ammonia, and sodium phosphate.

PHOSPHORUS.—Magnesium sulphate and ammonium chloride.

TANNIN.—Ten per cent ferric chloride.

Results

OVERWINTERED CANES.—In March and April, Dorothy Perkins canes brought in from the garden contained much more reserve starch than American pillar canes. The starch was most abundant

at the nodes and decreased slightly from the base of the canes upward. In those of Dorothy Perkins the cells of the primary and secondary medullary rays, of the primary xylem parenchyma, and of the outer pith and bud gap were filled with starch. The small active cells of the inner pith were filled with starch at the nodes and partially filled in the internodes. The endodermis sometimes con-

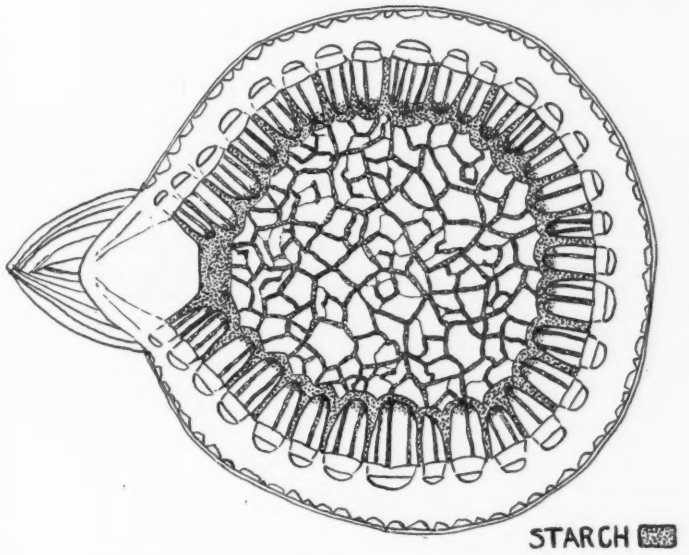


FIG. 4.—Diagram of transverse section of stem of Dorothy Perkins rose, showing arrangement of tissues and location of starch.

tained starch (fig. 4). In American pillar stems some of the medullary ray, outer pith, and bud gap cells were partially filled with starch, and the small cells of the inner pith contained a few grains each at the nodes, but were free from starch in the internodes.

There was no fructose and very little glucose in either rose. Other reducing substances (dextrin-like substances and tannins possibly) were usually present in abundance in both roses. The pith and cortex of the bud contained more of these substances than the pith and cortex of the cane. The pith of the cane sometimes con-

tained more near the bud than on the side opposite the bud. The embryonic region, leaf primordia, and procambial strands of the bud were usually free from reducing substances.

Those proteins which give the biuret reaction were present in the meristematic tissues of the bud and in the cambium and inner phloem of the cane. The cells of these tissues were filled with cytoplasm, and contained very large nuclei with large nucleoli. The pith and cortical cells of the bud also contained much protoplasm. The cells of the cambium and phloem parenchyma of the cane were high in content of protoplasm; the cells of the cortical parenchyma were lined with cytoplasm containing chloroplasts; while the small pith cells, medullary ray, and bud gap cells contained little protoplasm. The large pith cells were empty.

Tannins occurred very abundantly in the epidermis, sclerenchyma, scattered cells of the cortical parenchyma, endodermis, and in some of the cells of the bud gap and outer and inner pith.

Sphaero-crystals of calcium oxalate were more abundant in American pillar than in Dorothy Perkins. They were present in largest amounts in the pith of the bud and in the bud scales. The cortex and pith of the cane contained a considerable amount. Single crystals, probably also mostly calcium oxalate, were present in rows of cells in the sclerenchyma, collenchyma, and phloem of the cane.

The canes collected March 15, 1926, were made into cuttings and placed in water in saturated air (series 1). The changes in the content of starch and reducing substances in this series are given in table I.

All buds along the canes, except those under water, developed rapidly. As the buds grew, the reserve starch disappeared from the canes in the following order: (1) from the small cells of the pith in the internodes; (2) from the small cells of the pith at the nodes, beginning on the side opposite the bud; (3) from the outer pith and primary xylem parenchyma, beginning opposite the bud; (4) from the medullary rays, beginning opposite the bud; (5) from the bud gap.

After a few days in saturated air, a swelling appeared on the lower side at the base of the new shoots produced by cuttings of Dorothy Perkins. It usually continued to develop around the base,

TABLE I

CHANGES IN STARCH AND REDUCING SUBSTANCES IN CUTTINGS OF DOROTHY PERKINS AND AMERICAN PILLAR ROSES; SERIES 1, 1926

DATE	CONDITION OF MATERIAL		STARCH		FRUCTOSE		GLUCOSE		TOTAL REDUCING SUBSTANCES	
	Dorothy Perkins	American pillar	Dorothy Perkins	American pillar	D.P.	A.p.	Dorothy Perkins	American pillar	Dorothy Perkins	American pillar
March 15.....	B 2–3 mm. dormant	B 4–5 mm. dormant	MR+++++ BG+++++ P+++++	MR+++ PN++ PI*	N* I*	N* I*	N++ I+	N+ I+	N+++++ I++++	N++++ I++++
March 18.....	B 8–9 mm.	B 7–8 mm.	MR+++++ BG+++++ P+++++	MR++ PN+	N*	N*	N+++	N++++	B+++++	N+++++
March 20.....	.....	.....	MR+++++ BG+++++ P++++	MR++ PN+	N*	N*	N++++ (near B) B++++	N+	B+++++	B++++
March 22.....	Br 16 mm.	Br 10–15 mm.	MRN++++ PN+++	MR+ OP+ (near Br)	.....	.....	P++++	.....	N+++++ Br+++++	N++++ Br++++
March 26.....	R just visible	R none	MR++++ (near Br) P*	MR+ P*	N+	N+	PN+++ PI++	PN++ PI++	N+++++ Br+++++	Br++++
March 29.....	Br 18 mm. L 4	Br 10–15 mm. L 3	MR++++ (near Br)	MR+ (near Br)	N*	N+	P+++	P++	N++++	N+++
April 1.....	Br 30 mm. R 3–5 mm.	.....	MR++ OP++	.....	N*	N*	P++++	P+	N++++	N+++
April 6.....	R 6–8 mm.	.....	MR++ (near Br) BG+ P*	MR* BG+	N*	N*	Br++++	.....	N++++	N+++
April 8.....	R 10 mm.	L 5 R none	MR+ BG++	MR* BG*	.....	.....	.....	.....	N+++ Br++++	N+++ Br++++
April 15.....	Br 25–35 mm. L 4 R 10 mm.	Br 30 mm. L 7 R none	MR+ OP+ BG++	N*	N+	N+	N++	N+	N++++ Br++++	N+++ Br+++

Letters show position on stem of materials tested: B, bud; BG, bud gap; Br, branch; I, internode; IP, inner pith; L, leaf; MR, medullary ray; OP, outer pith; P, pith; N, node; R, root. The symbol (*) and plus marks (+) show amount of storage material present: *, trace to none; +, very little; ++, little; +++, moderate amount; ++++, considerable; +++++, excessive amount.

forming a collar-like enlargement (fig. 5). Roots appeared from this enlargement after eleven days in saturated air. At this time no enlargement and no root primordia had appeared in the young shoots of American pillar cuttings.

At the time roots appeared, Dorothy Perkins canes still had much starch, only that in the cells of the inner and outer pith having

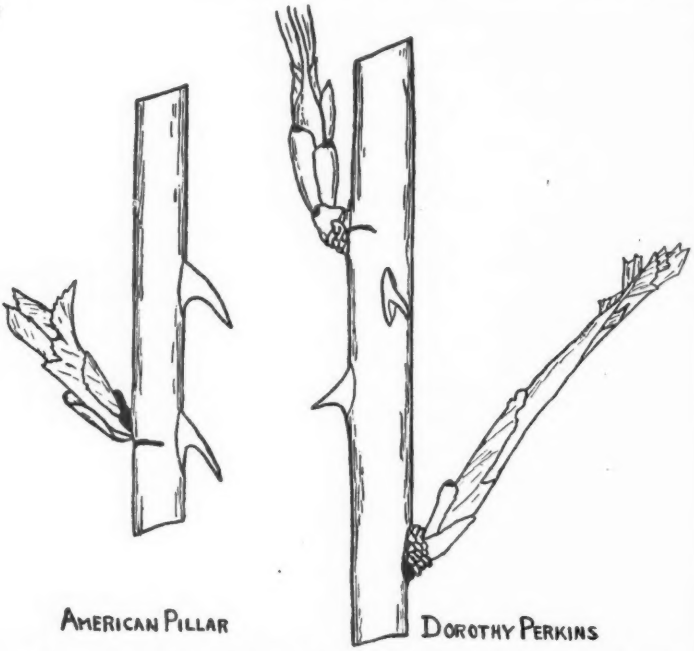


FIG. 5.—Cuttings of American pillar and Dorothy Perkins roses after 8–10 days in saturated air; roots appearing from the swelling at bases of shoots of Dorothy Perkins.

been hydrolyzed; while American pillar had lost all but a few grains in a few cells of the medullary rays, outer pith, and bud gap.

By the end of the experiment the new shoots of Dorothy Perkins were 2.5–3 cm. long, and the roots were 7–10 mm. long; while shoots of American pillar were 3 cm. long and had no roots. Dorothy Perkins still had starch in cells of the medullary rays, outer pith near

the branch, and in the bud gap; whereas all starch had disappeared from the tissues of American pillar.

No fructose was found in either rose at the beginning of the experiment. The glucose content varied in different canes, but there was usually a small amount present. After being placed in humid air the amount of glucose increased in Dorothy Perkins cuttings, and remained high until near the end of the experiment, when it dropped somewhat. Glucose was usually most abundant in the pith of the cane and young shoot. It was sometimes present in the cortex of the cane, with generally more on the side near the branch than on the opposite side. Often there was a considerable amount in the vascular cylinder, especially in the xylem vessels of the young shoot, but practically none was found in the meristematic tissues.

In American pillar cuttings the amount of glucose increased during the first few days in moist air, then decreased and remained low throughout the rest of the experiment. Glucose was high while the starch content was high, and dropped off when the starch content became low.

Reducing substances, not sugars, were present in large amounts in both roses throughout the experiment. Dorothy Perkins sometimes had more than American pillar. These substances were found chiefly in the pith of the branch and cane and in the vascular tissue.

These statements regarding reducing substances are based on the time required for reduction of copper tartrate. The osazone test, which was always made at the same time, was consistently negative for reducing sugars. This may have been due to the fact that the concentration of sugars was too low for osazone crystal formation, or to the fact that the presence of some substance (or substances) prevented the reaction. Later in the year, when the sugar content of the canes from the garden was much higher, the osazone test was positive.

The tests for nitrates were negative for both roses throughout the experiment.

Potassium was always abundant in all tissues of both roses, especially in the phloem, in the meristematic tissues, and in the pith of the branch.

The amount of calcium oxalate did not change noticeably in

either rose during the experiment. When the buds grew, the large sphaero-crystals of calcium oxalate in the pith remained at the very base of the young branches. Soluble calcium compounds were abundant in the young branches of both roses.

Proteins increased in the developing branches, but no decrease in protein content was detected in the canes. The enlargement at the base of young branches of Dorothy Perkins cuttings was due to an extraordinary development of phloem parenchyma. This tissue was high in protein content. The root primordia were first detected in this new tissue as small regions of cells which gave a pinkish color with the biuret reaction.

When kept for a few days in saturated air a dense precipitate formed with absolute alcohol in certain cells of the pith and cortex of the new shoots and in the small cells of the pith of the cane. This precipitate was not identified. After 10 or 11 days asparagin appeared in great abundance in both roses in those cells which had formerly contained the substance precipitated by alcohol. Asparagin remained high until the end of the experiments.

All of the later series gave results similar to those of series 1. In the spring of 1927, however, the difference in the starch content of the two roses was less striking than in 1926, but Dorothy Perkins again contained more than American pillar.

Fig. 6 shows the comparative changes in the starch content of the two roses in series 6. It will be seen that Dorothy Perkins canes contained considerably more starch than American pillar at the beginning of the experiment. Starch began to decrease in the canes of both roses when they were placed in humid air. In ten days roots appeared at the bases of the new shoots of Dorothy Perkins, but no roots developed on American pillar branches. On April 16, when shoots of Dorothy Perkins cuttings rooted, the canes still contained much starch at the nodes, while American pillar canes contained only a trace. At the end of the experiment, Dorothy Perkins still had a little starch, but American pillar had none.

In series 3, cuttings of both roses were placed under bell jars, some of which were covered with black paper. The temperatures in the bell jars, covered and uncovered, were the same at a given time, but varied with the room temperatures. Practically every new shoot

FIG. 6.—Diagrams showing comparative changes in reserve starch of Dorothy Perkins (DP) and American pillar (Ap) cuttings during series 6; roots visible in Dorothy Perkins cuttings on April 16.

of Dorothy Perkins rooted in nine days, in light and in darkness; while no American pillar shoots had rooted in 18 days, when the experiment was discontinued. The changes in reserves were the same in darkness as in light.

In the spring the rooting behavior was the same, whether canes were cut into pieces or left on the plant. Canes attached to the plants were placed parallel to each other on the ground and layered with moist peat moss for a distance of about 20 inches in the middle region. The buds, which were only slightly swollen on March 28, when the experiment was started, developed normally into flower branches. On June 24 each new branch of Dorothy Perkins had a good root system at its base, while no American pillar branches produced roots.

SUMMER CANES.—The composition and rooting behavior of new canes of both roses were studied during one season. During the summer the canes elongated very rapidly. Starch was present only in the endodermis, reducing substances were high, no nitrates were detected, and calcium oxalate crystals appeared early in the pith and scales of the newly formed buds. No marked differences in the composition of the two roses were noted during their early period of growth.

American pillar canes ceased their rapid growth in September, but Dorothy Perkins canes continued to elongate until November. Starch began to accumulate in the cells of the medullary rays and outer and inner pith as growth became slower. Deposition proceeded from the base of the canes upward. The starch content of American pillar canes reached its maximum during December and then began to decrease. The maximum starch accumulation in Dorothy Perkins occurred in January and February.

Reducing sugars were more abundant in the canes while starch was accumulating than at any other time. The osazone test showed that there was an abundance of fructose and glucose in the pith, xylem, and phloem, with more at the nodes than in the internodes. After the maximum in starch accumulation had been reached, the reducing sugars were low.

New canes were made into cuttings at intervals during the summer and fall, and were treated in the same way as the overwintered canes. The upper one or two buds of each cutting developed into

branches, but the other buds remained dormant. Cuttings of both roses taken in July and August rooted from the bases of the cuttings, but not from the bases of the new shoots. As already stated, the starch content of both roses was very low during this time. Cuttings taken in the fall, when starch was accumulating in the canes, rooted from the bases of the developing branches and not from the canes. Four of eight branches of American pillar cuttings rooted, and all of eight branches of Dorothy Perkins cuttings rooted. American pillar branches produced one to four roots each, while Dorothy Perkins branches produced seven to twelve roots each. In all cases the rooting required two months for American pillar and one month for Dorothy Perkins. Unfortunately the number of cuttings used in this experiment was too small to make the results conclusive.

Experiments were performed between December and March to determine the effect of temperature on the rooting of cuttings in saturated air. Cuttings consisting of two or three nodes each were placed in constant temperature ovens at 32°, 25°, 20°, 15°, 10°, and 5° C. for one month. The results are shown in table II. As reported by ZIMMERMAN (14), rooting occurred throughout a wide range of temperature, and the time required varied with the temperature. Dorothy Perkins rooted well from the bases of young branches at 25°, 20°, and 15°, more slowly at 10°, and poorly at 32° and 5°. Rooting was equally good on pieces from bases, middles, and tops of canes. A few American pillar cuttings taken from the bases and middles of old canes rooted from the bases of young branches at 25°, 20°, and 15° C.; none rooted at 32°, 10°, or 5°. Samples of the cuttings used in these experiments were examined for starch when collected. Those cuttings which rooted contained considerable reserve starch. In experiments with summer canes, as well as with overwintered ones, there seemed to be a correlation between starch content and rooting behavior.

So far, no differences in structure of the two roses which could clearly account for their difference in rooting behavior have been detected. American pillar canes are thicker and stiffer than Dorothy Perkins canes. This is due largely to the greater amount of xylem in American pillar. The pith of Dorothy Perkins is more compact than that of American pillar.

The branches are similar in structure during their early develop-

TABLE II

ROOTING OF DOROTHY PERKINS AND AMERICAN PILLAR ROSE CUTTINGS AT CONSTANT TEMPERATURES

	Temperature												Control in light room temperature	
	32° C.		25° C.		20° C.		15° C.		10° C.		5° C.			
	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted
	Experiment 1, December 15–January 12													
American pillar..	9	0	9	2	9	2	.....	.....	9	0	.....	.....	3	0
Dorothy Perkins.	6	0	6	6	6	6	.....	.....	6	3	.....	.....	6	6
	Experiment 2, January 15–February 21													
American pillar..	9	0	10	0	9	0	12	1	10	0	12	0	.....	.....
Dorothy Perkins.	10	0	11	1	9	8	13	12	12	9	12	5	.....	.....

ment, but after three or four days the cambium at the base of Dorothy Perkins branches becomes unusually active toward the outside, and produces an extensive region of parenchymatous phloem tissue. The adventitious roots originate in this tissue.

Discussion

New shoots on the overwintered canes of Dorothy Perkins rose send out roots while in air of high humidity, as well as when planted in sand or peat moss. Moisture, therefore, must be the primary factor which induces root growth in Dorothy Perkins if the temperature and oxygen supply are favorable. Rooting occurred throughout a wide range of temperature, and light did not seem to play a part. Layered canes rooted as well as cuttings; evidently separation from the mother plant was not an important factor.

On the other hand, new shoots arising from overwintered canes of American pillar rose did not root. Some factor or factors other than moisture must be involved with this species. The primary difference detected between the two roses was in the reserve starch storage. Dorothy Perkins contained much more starch than American pillar in the two successive spring seasons. Winter canes kept in saturated atmosphere send out new shoots which necessarily draw on the stored food supply. The starch first disappeared from the side opposite the bud, and finally the last trace was found at the base of the new shoot. American pillar rose exhausted its entire supply in 10–12 days, whereas Dorothy Perkins rose showed a moderate amount after three to four weeks. In a few cases American pillar rooted in the fall when its starch content was high, and both failed to root when they lacked starch reserve.

Preliminary experiments to increase the carbohydrate content of American pillar canes and to decrease it in Dorothy Perkins before testing their rooting have not yet been successful.

The relation between the reserves and the rooting behavior of cuttings has been reported by other investigators. KRAUS and KRAYBILL (3) found that cuttings of tomato which are old and yellow in color, high in carbohydrates, and low in total nitrogen and nitrates, root well in humid air. Green stems which contain starch and are fairly high in total nitrogen will root, but not so profusely

as the former; and green stems without starch reserves and very low in free reducing substances but high in total nitrogen and nitrate nitrogen will not root. This work was extended and confirmed by STARRING (13), REID (7, 8, 9), and SCHRADER (10), all working with tomato.

PRIESTLEY (6) states that in some cases the food supply largely controls the production of new root initials. He thinks that adventitious roots arise in the cambium, and that stems which have no true endodermis allow leakage of food out through the cortex to the superficial meristems, with consequently not enough food remaining in the cambium for the production of root initials. Some etiolated shoots produce an endodermis. These make little growth from the superficial meristems, but root with ease, since the nutrients are held by the endodermis and can be used by the cambium.

SMITH (12), working with *Coleus*, found that the condition of the carbohydrate reserves had a marked influence on rooting. Plants in full light, with reserve carbohydrates largely in the form of starch, rooted sooner and better than plants in the shaded greenhouse where reserves were mostly reducing sugars.

Rooting from the bases of Dorothy Perkins branches involves, first, a special activity of the cambium by which an extraordinary amount of phloem tissue is produced, and second, the change in certain cells of the new tissue from the parenchymatous to the meristematic condition, thus initiating root primordia. The principles involved in these processes are still largely a matter of theory. PEARSALL and PRIESTLEY (5) account for the transformation of tissues from a vacuolated, non-dividing condition into a non-vacuolated, or only slightly vacuolated, meristematic condition by the fact that they lie across a gradient of hydrogen-ion concentration. Along this gradient the principal proteins of some cells are at their iso-electric point. These cells will lose water, accumulate protoplasm, and become meristematic.

SMITH (11) states:

> Cell division is the expression of a series of catenary reactions depending upon the presence in a certain concentration and a certain ratio of carbohydrate and amino-radicles. Only when this carbon-nitrogen balance is maintained can a cell remain meristematic. Under normal conditions the carbohydrate is always

in excess, and it is this excess which determines cell-maturity and the cessation of new growth. If by any means the required C:N ratio can be restored, a mature tissue can be incited to regeneration.

Summary

1. Adventitious roots are produced in humid air on the new shoots from cuttings of Dorothy Perkins rose and not from like portions of American pillar rose.

2. Microchemical examination showed that Dorothy Perkins cuttings contained more reserve starch than American pillar cuttings. At the time of rooting, Dorothy Perkins had considerable reserve starch remaining, while during a similar period the reserve starch of American pillar was depleted.

3. The changes in the reserves were the same in both roses. As the starch in the canes was hydrolyzed, the content of reducing sugars increased, especially in the new branches. Asparagin was abundant in the branches after 8–11 days.

4. Only slight differences in the anatomy of the overwintered canes were noted. A swelling at the base of new branches of Dorothy Perkins was produced by an unusual development of secondary phloem. Adventitious roots were initiated in this region. These changes did not occur in American pillar branches.

5. Rooting from the bases of young shoots of Dorothy Perkins rose seemed to be related to the high content of reserve starch.

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GROWTH OF SEEDLINGS IN LIGHT AND IN DARKNESS IN RELATION TO AVAILABLE NITROGEN AND CARBON[1]

MARY E. REID

(WITH PLATES I–IV)

Introduction

The influence of reserve substances in the plant upon its subsequent growth involves a multitude of problems of great importance, both from a practical and from a theoretical standpoint. The present investigations are concerned with the influence of certain types of reserve foods found in seeds upon the development of the seedling. A study has been made concerning the relation to growth of the amount and nature of the reserves of carbon and nitrogen which an embryo plant has at its disposal.

Some of the more practical questions which have prompted the research are as follows:

What are the responses of seedlings having available different amounts of reserve carbon and nitrogen when extra amounts of carbon and nitrogen are supplied externally? Is fertilization with nitrogen advisable in the early growth of all types of seedlings, regardless of weather and light conditions and the nitrogen reserves of the seed? Is the seedling with the larger reserves of carbon better able to live and thrive during prolonged periods of dull, cloudy weather during the early growth of the plant? Is the seedling with the larger amount of stored nitrogen at its disposal better able to withstand conditions of drought or of poor soil during the early phase of development, when nitrogen from an outside source is unavailable or available in very limited amounts?

Although the results of these experiments do not furnish answers to all these questions, it is considered that they supply considerable additional data toward this end. Previous investigations (11) with

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tomato cuttings varying in the relative proportions of the reserves of carbohydrates and nitrogen had shown very different responses when exposed to different environmental conditions, such as variations in light and darkness and the presence and absence of nitrate nitrogen in the nutrient medium. It was found that: (1) An abundant reserve of carbohydrates favored the rapid assimilation of nitrates. This was in agreement with the observations of KRAUS and KRAYBILL (8) in their study of the growth of the tomato plant. (2) An abundant reserve of carbohydrates but limited supply of nitrogen favored the growth of roots, but not that of shoots. (3) An abundance of nitrogen and an abundance of carbohydrates favored the growth of both shoots and roots. (4) An abundance of nitrogen but a limited amount of carbohydrates favored growth of shoots, especially of leaves, but not growth of roots. (5) The assimilation of nitrates occurred both in light and in darkness, but more rapidly in light, and the assimilated products were used in favoring growth of shoots especially.

The present studies have dealt with the development during the seedling stage only, and have related primarily to the influence of varying amounts of nitrogen and carbon upon the growth of shoots and roots. Experiments have been performed in light and in darkness with no nitrogen obtainable except that present in the reserves of the seeds, and also with nitrogen available in the form of nitrates. The following types of seeds were used:

Little Club wheat Vermont Champion barley Giant Winter rye Illinois low-protein corn Hännchen barley (Dickinson, N.D.)	Low protein content; high starch; low fat	Relatively high content of gliadin and glutenin; amide N high; relatively small amount of globulin protein
Blue stem wheat Marquis wheat Hännchen barley (Aberdeen, N.D.) Illinois high-protein corn	Relatively high protein content; high starch; low fat	Relatively high content of gliadin and glutenin; high-protein corn lower in amide N and higher in globulin than low-protein type (SHOWALTER AND CARR, 14)

First of all peas Canada White peas New Era cow peas White Marrow bush beans Pekin soy beans	Moderately high protein content; moderately high starch content; low fat (except soy bean)	
Mammoth Russian sunflower Large Warted Hubbard squash Rocky Ford muskmelon Bonnie Best tomato	Very high protein; low starch; high oil	High globulin content; amide N low

Experimental methods

The seeds were sterilized by immersion in a 0.25 per cent solution of uspulun. Starchy seeds were left in the solution for one hour, and those of the oily type for one-half hour, after which they were rinsed in freshly distilled water. The seeds were placed in germinators between layers of moist filter paper and allowed to sprout before being planted. This precaution was necessary since some of the seedlings were to be grown without any nitrogen from an outside source, and consequently the possibility of the seedlings obtaining nitrogenous products by the decomposition of seeds which failed to germinate had to be avoided. When the radicles had attained a length of 0.5–2 cm. the seedlings were planted in pulverized quartz and contained in 7-inch clay bulb pots. Glazed porcelain dishes with rims an inch high were used as saucers. The pots containing the sand, together with the saucers, had previously been sterilized by heating for an hour in a steam sterilizer maintained at a pressure of 15 pounds. The cultures were moistened with nutrient solutions made with salts of tested purity, and prepared according to the following formulas:

Solution containing nitrates

A	B
2 per cent $MgSO_4$	4 per cent $Ca(NO_3)_2$
2 per cent KH_2PO_4	2 per cent $CaCl_2$
2 per cent KNO_3	1.5 per cent $CaSO_4$

Solution lacking nitrates

A	B
2 per cent $MgSO_4$	2 per cent $CaCl_2$
2 per cent KH_2PO_4	1.5 per cent $CaSO_4$
1 per cent KCl	

The solutions were diluted before applying them to the cultures. In preparing the solution containing nitrates, 100 cc. of solution A was made up to 1 liter with distilled water; 100 cc. of solution B was diluted in the same manner and the solutions A and B were then mixed. The solution lacking nitrates was diluted similarly. The cultures were given fresh solutions every second day in some experiments, and every third or fourth day in others. The time for making fresh applications was determined largely by light and temperature conditions, which affected the rate of growth and the ability of the seedlings to utilize the mineral nutrients. The same procedure was followed throughout the course of any one experiment. The level of the solutions in the saucers was kept nearly constant between times of applying nutrient solutions by the addition of distilled water. Freshly distilled water was used at all times. This precaution was considered necessary, since it has been shown by SEIBERT (13) that certain kinds of microscopic organisms may thrive in distilled water tanks and pipes, and that these organisms have the ability to fix atmospheric nitrogen. The external nitrogen supply was controlled in these cultures, except that ammonia in the atmosphere was not eliminated as a factor. However, all seedlings in any experiment were subjected to the same atmospheric conditions with reference to this factor, and hence it is unlikely that it can be responsible to any extent for the differences in growth that are here described. Experiments have been conducted with seedlings grown in darkness and in light with each of the two types of solutions. Except in one case (March 5–26, 1926) all experiments were repeated, and the results of the second test were found in general to be in close agreement with those of the first.

Growth of seedlings in darkness

The question of etiolation has enlisted the interest of a number of physiologists, whose investigations on the problem were relatively extensive in the botanical literature of fifty to sixty years ago. SACHS, G. KRAUS, GODLEWSKI, STEBLER, PFEFFER, BATALIN, RAUWENHOFF, and VINES made important contributions at this time. Still earlier, DECANDOLLE (2) had described the characteristics of the etiolated plant, and attempted, as have many others

since, to account for the peculiarities of growth in darkness. The chief characteristics of the etiolated plant as mentioned by these earlier investigators may be said to be a greatly elongated stem, and leaves much reduced in size. Cotyledons which in the light grow and develop into foliage leaves remain small and undeveloped in darkness. Of all the peculiarities of the etiolated seedlings perhaps the absence of chlorophyll is the most outstanding. Almost no mention is made of the characteristics of the roots of etiolated seedlings.

The results of some of SACHS' (12) experiments show that the great elongation of the stem and reduction in size of the leaves of etiolated plants is not without exceptions. The leaves of many monocotyledonous plants become longer in darkness than in light. Also the leaves of certain dicotyledonous plants, as *Beta*, become almost as large when grown in darkness as in light. SACHS postulated that the reason leaves failed to develop in darkness was not because of lack of food (carbohydrates supposedly), but was due to some unknown influence of chlorophyll.

DE SAUSSURE (3) was probably the first to state that leaves are dependent for their growth on the products of their own assimilation (carbohydrates inferred). G. KRAUS (9) was also of this opinion, and claimed that the leaf can only develop in darkness to the stage where it can begin to assimilate if it receives the light; if it cannot assimilate it will remain small and soon die. His only proof, however, for the fact that assimilation is essential for the growth of leaves is that he did not find starch in etiolated leaves. He recognized the fact that non-nitrogenous reserve materials as fats may be present in cotyledons, and yet the cotyledons do not grow beyond a certain relatively small size in darkness. He considered that deficiencies in cell wall formation were the chief limiting factors, therefore, and that light is necessary for the transformation of materials into cell walls. His own work (9) and that of RAUWENHOFF (10), and of a number of later investigators, have shown that light has a marked influence on the thickening of cell walls and the maturation of tissues. G. KRAUS and RAUWENHOFF thought the effect was produced by the processes of synthesis and utilization of carbohydrates. GODLEWSKI (5) claimed that the influence of light on the thickening of cell walls and its growth-retarding effect on the

growth of stems have nothing to do with the assimilation of carbohydrates. VINES (16) had previously presented data which were interpreted as proving also that the retarding influence of light on the growth of leaves is completely independent of assimilation. Both of these investigators referred to the need of uniform light for the growth of stem and leaf, rather than to the effect of strong light as compared with weaker light.

Although most of the research as to the effects of light and darkness on the growth of different organs has been conducted with seedlings, not much attention has been paid to the influence of the amount and nature of the food reserves of the seed upon the type of growth. Nevertheless such a relation was partially recognized by the investigators who studied somewhat the chemical content of the seedlings. Descriptions of the growth responses are very incomplete, however.

It has not been the province of this research to enter into a detailed study of the phenomena of etiolation, but rather to obtain some quantitative measurements of the growth of different organs, to be used for comparison with results of subsequent experiments with seedlings grown in the light.

1. SEEDLINGS GROWN WITH NO EXTERNAL SOURCE OF NITROGEN

The seedlings of each kind were allowed to grow until the shoots attained their maximum length. Several preliminary tests were made to determine the most practicable method. Measurements of the heights of certain plants in each culture were made at intervals as the time of attaining maximum size approached. When no further elongation of the shoot occurred the experiment was terminated. The general appearance of the plant was taken into consideration, and slight indications of shrinking or wilting of the leaves were likewise used as an index that there was cessation of growth. The dark room in which the seedlings were grown was maintained at a nearly uniform temperature (21° C.), and favorable conditions of atmospheric moisture were obtained by evaporation of moisture from the cement floor covering the pebbles which was sprinkled with water daily.

In harvesting the seedlings, the roots were carefully washed

free of the quartz sand and rinsed in distilled water. Measurements of the lengths of roots and stems and of length and width of leaves and cotyledons were obtained, and also the green weights of the tissues. The quantitative results are given in table I, and illustrations of cow pea, soy bean, muskmelon, tomato, sunflower, and high- and low-protein corn seedlings are shown in figs. 1–7. Due to the fact that so many different kinds of seedlings were grown in these experiments, it was not possible to preserve the material for dry weight determinations.

Seedlings which grew from high-protein, high-oil seeds had the highest shoot to root ratios. This largely results from the development of an exceedingly long stem and a small amount of roots. The stems have a very high water content and a relatively small amount of protoplasm in the cells. Some of the seedlings of this group (for example, squash) may produce one leaf which remains very small, or no visible leaves, as in the case of tomato. Sections of the incompletely developed cotyledons showed that the tissues have a very dense compact structure. The cells remain small and intercellular spaces are only slightly developed. The similarity in behavior of these seedlings, representing three different families, suggests that the type of growth is related to the nature and quantity of reserve foods which are available.

The smallest weight of shoots in proportion to roots was found among the Gramineae. The low ratio results from the relatively greater weight of roots, the small size or lack of stems, and the extensive leaf development in which a large amount of the reserve materials has been used. The leaves of these seedlings have a much higher protoplasmic but lower water content than had the stems of the high-protein dicotyledonous seedlings previously described. Seedlings of the grass family have leaves longer and narrower, but thinner in cross-section, than those of similar seedlings grown in the light in normal atmosphere. This difference in length of leaf in light and in darkness results chiefly from the greater length of the leaf sheaths in the case of seedlings grown in darkness. The leaf blades of the high-protein types of the grass family tend to be longer in the light than in darkness, but those of the low-protein types tend to be longer in darkness than in light. Microchemical tests for starch

TABLE I

SEEDLINGS GROWN IN DARKNESS WITHOUT NITRATES, MARCH 7–APRIL 7, 1924

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	WEIGHT OF SEEDS (GM.)	TOTAL NITROGEN IN SEEDS (%)	AVERAGE LENGTH		GREEN WEIGHTS PER PLANT			RATIO OF WEIGHTS OF SHOOTS TO ROOTS	TOTAL GROWTH PER GM. OF RESERVE FOODS (GM.)
						Shoots (cm.)	Roots (cm.)	Shoots (stems + leaves) (gm.)	Roots gm.	Leaf blades + cotyledons (gm.)		
Wheat	Blue stem	60	17	2.32	2.27	24.0	8.5	0.151	0.068	0.084	2.22	5.6
Wheat	Marquis	60	16	2.08	2.50	27.5	8.9	0.166	0.054	0.086	3.04	6.3
Wheat	Little club	60	16	1.99	1.52	20.8	15.2	0.158	0.066	0.087	2.39	6.7
Barley	Vermont champion	60	15	1.59	1.82	21.5	10.2	0.135	0.062	0.073	2.19	7.4
Oats	Storm king	60	14	1.83	2.68	21.9	11.8	0.150	0.086	0.078	1.75	7.6
Rye	Giant winter	60	15	1.37	1.75	19.0	9.7	0.113	0.052	0.071	2.19	7.2
Rice	Honduras	60	20	1.41	1.68	34.0	5.6	0.095	0.031	0.020	3.08	5.3
Corn	Illinois high-protein	12	14	3.27	2.76	36.0	23.7	1.470	0.686	0.696	2.14	7.9
Corn	Illinois low-protein	12	14	4.69	1.09	29.2	28.0	1.290	0.750	0.575	1.72	5.2
Corn	Illinois high-oil	12	15	3.27	.....	32.9	25.8	1.402	0.772	0.728	1.81	8.0
Corn	Illinois low-oil	12	15	7.31	.....	36.1	27.0	1.800	0.950	0.706	1.90	4.5
Peas	First of all	36	19	6.26	4.11	40.5	9.3	1.121	0.397	0.067	2.82	8.7
Peas	Juno	36	20	9.46	5.10	29.0	9.3	1.268	0.513	0.131	2.47	6.7
Beans	White Marrow bush	30	16	9.99	3.85	51.3	13.2	1.993	0.526	0.111	3.78	7.6
Cow peas	New era	26	13	3.39	4.40	32.1	9.6	0.757	0.237	0.058	3.19	7.6
Soy beans	Peking	26	26	1.75	3.40	.....	.....	0.642	0.172	0.074	3.72	12.1
Sunflower	Mammoth Russian	18	17	0.85	4.56	27.2	6.7	1.020	0.105	0.165	9.71	23.8
Squash	Hubbard Warted	14	19	2.04	5.10	28.6	13.0	2.867	0.432	0.520	6.63	23.1
Melon	Red Rocky Ford	20	19	0.26	5.62	14.0	7.0	0.251	0.040	0.040	6.25	22.4
Tomato	Bonnie Best	33	15	0.08	.....	10.1	3.2	0.040	0.005	0.007	7.26	18.8

and free-reducing substances, made on tissues of some of the seedlings just previous to harvesting, showed that many of the seedlings contained little or no starch except in the guard cells of the leaves and occasional grains in the bundle sheaths at the time the seedlings stopped growing. However, some of them contained small amounts of reducing substance.

Seeds of the Leguminosae are in certain respects intermediate to the other two groups in the chemical composition of the reserve foods, and the seedlings have a tendency to be intermediate in their responses with respect to the relative proportions of shoots to roots. Several types of seedlings of this group (for example, two kinds of peas) stopped growing before the reserves from the cotyledons were depleted. Microchemical tests showed that very little reducing substance could be found in the seedlings or in the cotyledons, but that a small amount of starch was left in the cotyledons. The supply of soluble carbohydrates in the seedling may have been insufficient to provide for further growth. It has been shown by a number of investigators that leguminous seedlings grown from high-protein seeds of especially high-protein content have insufficient carbohydrate reserves to allow a complete utilization of the stored nitrogen. The possibility of some limiting factor other than a carbohydrate should also be considered. No tests were conducted to determine whether the seedlings could be kept growing for a longer time by immersing the roots in sugar solutions.

Some variations in the relative weights of shoots and roots in relation to differences in temperature have been observed. In one series of experiments with high- and low-protein corn it was noticed that a relatively greater weight of roots was produced at 27° than at 20° C.

Observations made on cross-sections of stems and roots of the dicotyledonous seedlings showed that the processes of both nuclear and cell division have been much limited. Secondary thickening is less advanced than in stems and roots of similar seedlings grown in the light. Some microchemical tests for cell membrane substances have been made. There is some evidence that lignin is present in the xylem vessels of the stem in most of the types of seedlings. Darkness does not prevent the deposition of strengthening materials in the cell walls, although it does limit the process greatly.

II. Seedlings grown with nitrates in nutrient solution

The quantitative data are presented in table II. When nitrates were obtainable by the growing seedlings, there was found to be a definite increase in the total green weight in thirteen of the nineteen types studied. Two of the types had the same total green weight when nitrates were available as when they were not. A decrease in the total green weight of four types was noted. Possibly in the latter forms carbohydrates were a limiting factor. There is evidence from some of the experiments to be described in a subsequent paper that the presence of nitrates in the nutrient solution stimulates respiration. This alteration in the rate of respiration of seedlings grown in darkness would have considerable effect on the duration of the reserve carbohydrates and fats.

The increases in total green weights of tissues with the use of nitrates are not nearly so great as has been found in the experiments with tomato cuttings, which had a much more abundant store of reserve carbohydrates. It was found that nitrates favored the growth of shoots much more than of roots in the experiments both with seedlings and tomato cuttings. In some of the experiments with cuttings, in which the nitrogen and carbohydrate reserves were both moderately high, the presence of nitrates in the nutrient solution in which the cuttings were grown had a definitely unfavorable effect on the growth of roots. Only four of the nineteen types of seedlings had growth of roots increased by the presence of nitrates in the nutrient solution; thirteen types had root growth somewhat suppressed; and two types had about the same weight of roots with nitrate as without. Growth of shoots was definitely favored in fourteen types of seedlings; it was inhibited in four types; and about the same in two types. These responses as to the stimulating effect of nitrates on growth of shoots also agree with results obtained with tomato cuttings.

Summary of results obtained with seedlings grown in darkness

1. There appears to be a tendency for the relative weights of shoots to roots to vary according to the carbohydrate and nitrogen content of the seed: the higher the supply of carbohydrates in pro-

TABLE II

SEEDLINGS GROWN IN DARKNESS WITH NITRATES AVAILABLE, MARCH 9–APRIL 7, 1925

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	WEIGHT OF SEEDS (GM.)	TOTAL NITROGEN IN SEEDS (%)	AVERAGE LENGTH		GREEN WEIGHTS PER PLANT		RATIO OF WEIGHTS OF SHOOTS TO ROOTS	TOTAL GROWTH PER GM. OF RESERVE FOODS (GM.)
						Shoots (cm.)	Roots (cm.)	Shoots (gm.)	Roots (gm.)		
Wheat	Blue stem	40	17	1.55	2.27	26.7	9.2	0.196	0.055	3.58	6.4
Wheat	Marquis	40	17	1.39	2.50	28.0	9.7	0.173	0.047	3.68	6.3
Wheat	Little club	40	17	1.33	1.52	24.0	9.2	0.180	0.077	2.32	7.7
Barley	Vermont champion	20	18	0.53	1.82	23.6	10.2	0.188	0.079	2.38	10.1
Oats	Storm king	31	12	0.94	2.68	25.3	9.2	0.144	0.074	1.94	7.2
Rye	Giant winter	52	17	1.19	1.75	22.2	10.1	0.155	0.053	2.91	9.1
Rice	Honduras	38	24	0.89	1.68	30.4	9.2	0.117	0.019	6.19	5.8
Corn	Illinois high-protein	12	22	3.27	2.76	37.1	14.8	1.340	0.427	3.13	6.5
Corn	Illinois low-protein	12	22	4.69	1.09	43.0	21.1	1.750	0.510	3.42	5.8
Corn	Illinois low-oil	12	22	7.30	.....	40.5	15.2	1.480	0.377	3.92	3.0
Peas	First of all	23	24	4.00	4.11	53.9	8.9	1.360	0.345	3.94	9.8
Peas	Juno	23	24	6.04	5.10	52.2	10.1	1.250	0.394	3.17	6.2
Beans	White Marrow bush	9	24	2.99	3.85	46.2	8.8	2.593	0.483	5.36	9.2
Cow peas	New era	20	23	2.61	4.40	28.5	9.3	0.725	0.177	4.08	6.9
Soy beans	Peking	19	29	1.28	3.40	46.2	5.1	0.822	0.098	8.35	13.6
Sunflower	Mammoth Russian	10	17	0.47	4.56	29.2	8.9	1.180	0.112	10.50	27.5
Squash	Hubbard	9	29	1.31	5.10	32.6	12.2	4.793	0.676	7.08	37.6
Melon	Red Rocky Ford	18	18	0.24	5.62	13.5	3.9	0.264	0.035	7.43	22.5
Tomato	Bonnie Best	46	18	0.12	?	12.7	1.8	0.061	0.004	16.08	25.0

portion to the nitrogen, the lower the relative weights of shoots to roots. The relation is probably not so clear as it might be if the carbohydrate reserve were not a limiting factor to the utilization of nitrogen in some of the high-nitrogen types of seedlings.

2. Nitrate nitrogen is assimilated by seedlings grown in darkness. Variations in ability to synthesize nitrates into growth-promoting substances are doubtless caused to some extent by differences in the carbohydrate or fat reserves, or in some cases by differences in both fats and carbohydrates.

3. Nitrates tend to increase growth of shoots in darkness, but they inhibit growth of roots somewhat, especially if the supply of reserve carbon compounds is much limited.

4. There is a marked inhibition in growth as to size and number of leaves in all types of seedlings investigated except among the representatives of the Gramineae. Observations indicate that this is due partly to a failure of the cells already formed to grow, although there is also a limitation of cell division. In general, the greater compactness of the foliaceous tissues, lack of intercellular spaces, and the small size of cotyledons of the foliaceous type (as in squash) show that growth of cells is inhibited.

5. Growth of roots is much restricted in practically all types of seedlings, but stem elongation is extensive with the exception of that of seedlings of the Gramineae, in which the leaf sheaths are much elongated.

6. The processes of secondary thickening of stems and roots and of deposition of materials in the cell walls are very much inhibited.

Seedlings grown in light in normal atmosphere

Seasonal differences have been found to modify noticeably the growth of seedlings in the light. An attempt has been made to avoid differences caused by changes of season, such as length of day, temperature, and intensity and quality of light. The experiments on the effect of nitrogen starvation, and nitrogen feeding on the growth of different types of seedlings in the light, have been conducted chiefly during the months of October and March. During these months day length is approximately the same, except for the fact that in the October experiments the days became shorter as the experiment progressed, and in March they became longer. How-

ever, since most of the seedlings did not grow for more than three weeks, this difference in length of day at the beginning as compared with the end of the experiment could not have had much influence on the responses.

In the few tests that have been made during the months with longer days, in which the sunlight was also more intense, lower shoot to root ratios were obtained. This is to be noted to some extent by a comparison of the results shown in tables III, IV, VII, and VIII. Although the experiments were carried on during corresponding spring and fall months of the year, in the October experiment the weather was cloudy about half of the time; whereas during the March experiment there was a larger proportion of hours of sunshine. In some experiments conducted during November and the early part of December, it was noted that the shoot to root ratios were considerably higher than in the October experiment and much higher than in the March experiment.

Experiment 1, October 1925

Two sets of cultures were prepared in the manner described for seedlings grown in darkness. One set was given the solution lacking nitrates and the other the solution containing nitrates. The experiment was terminated when the seedlings had grown to maximum size, which was determined partially by the time of exhaustion of the food reserves, and partially by observing the time at which the shoots ceased growing. It was found that different kinds of seedlings require different periods of time to reach maximum size. The seedlings of the grass family, especially the low-protein types, attained their development in the shortest time. The cultures receiving nitrates were always harvested on the same day as the corresponding cultures not receiving extra nitrogen.

The temperature of the greenhouse was kept at 21°–22° C. during most of each 24-hour period, but on sunshiny days there was a rise during the middle of the day. All seedlings in each of the two sets of cultures were grown under the same external conditions, so such differences as are noted in the responses must be due to differences in the nature and amount of the food reserves and to the hereditary factors.

1. Seedlings grown without extra nitrogen

Table III gives the quantitative results of this experiment, and figs. 8–13 present illustrations of soy bean, cow pea, muskmelon, sunflower, and low- and high-protein corn seedlings. The ratios of the weights of shoots to roots were much lower than those of corresponding seedlings grown in darkness; but in agreement with the results obtained in darkness, the higher protein types of seeds produced seedlings with higher shoot to root ratios. The low-protein types, such as are found in the Gramineae, developed seedlings with the lowest shoot to root ratios. The high-protein, high-oil seeds yielded seedlings with the highest shoot to root ratios; and, as in the case of the experiments in darkness, seedlings of the Leguminosae were intermediate in their responses.

All seedlings in this experiment had an extensive development of roots in proportion to the size of the shoots. Early in the growth of the seedlings starch began to accumulate in certain tissues. The greatest ability to store starch was noted in tomato, squash, and muskmelon seedlings. It was previously shown by KRAUS and KRAYBILL (8), and later by others, that carbohydrates rapidly accumulate when nitrogen is a limiting factor for protein synthesis and growth. The non-nitrogenous carbon compounds accumulate in sunflower seedlings in the form of oils, although considerable starch is also present. In seedlings of the grass family a relatively large amount of the carbohydrate material appears to be deposited in the cell walls. All tissues of these seedlings undergo rapid differentiation, and the cell walls of the strengthening tissues rapidly increase in thickness. The tissues are physiologically old when the seedlings have grown for only two or three weeks, and the lower the nitrogen content of the seed the more rapid the maturing and eventual senescence of the tissues.

After emerging into the light, the cotyledons and leaves as they grew developed an intense green color. In some cases the color was of a darker and somewhat bluer green than that observed in the plants receiving nitrates. During the latter part of the growth period the color gradually became less intense and developed more of a yellowish tinge. These results as to amount of chlorophyll in leaves and cotyledons of seedlings receiving and not receiving nitrates

TABLE III

SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE WITH NO EXTERNAL SOURCE OF NITROGEN, OCTOBER 7, 1925

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	TOTAL NITROGEN IN SEEDS (%)	AVERAGE LENGTH OF			GREEN WEIGHT PER PLANT			RATIO OF WEIGHTS OF SHOOTS TO ROOTS
					Shoots (cm.)	Roots (cm.)	Leaves (cm.)	Shoots (gm.)	Roots (gm.)	Leaves (gm.)	
Wheat	Blue stem	53	14	2.27	17.0	19.2	13.2	0.146	0.198	0.101	0.73
Wheat	Marquis	53	13	2.50	15.5	16.3	11.6	0.142	0.199	0.103	0.71
Wheat	Little club	56	13	1.52	10.7	12.8	7.7	0.096	0.140	0.053	0.64
Barley	Vermont champion	43	14	1.82	11.2	17.0	9.4	0.128	0.198	0.099	0.65
Oats	Storm king	46	18	2.68	21.0	10.9	14.5	0.171	0.268	0.115	0.64
Oats	Clydesdale	30	15	2.82	11.2	10.3	7.8	0.142	0.254	0.097	0.55
Rye	Giant winter	44	15	1.75	10.5	16.5	7.8	0.086	0.091	0.057	0.45
Rice	Honduras	44	22	1.68	13.2	10.5	8.6	0.056	0.069	0.023	0.81
Corn	Illinois high-protein	10	17	2.76	24.2	14.2	18.0	1.472	1.540	0.838	0.95
Corn	Illinois low-protein	10	17	1.09	14.2	11.7	9.5	0.634	0.800	0.336	0.79
Corn	Illinois high-oil	10	19	.....	23.5	20.0	17.0	1.312	1.775	0.740	0.73
Corn	Illinois low-oil	5	19	.....	20.5	15.2	15.2	1.600	1.952	0.812	0.82
Peas	Juno	18	33	5.10	17.0	12.4	2.4	1.936	1.717	.....	1.12
Peas	Canada white	16	23	3.60	23.7	12.1	2.4	0.947	0.890	0.456	1.06
Beans	White Marrow bush	5	18	3.85	15.7	11.2	6.5	3.018	1.544	1.754	1.94
Cow peas	New era	15	17	4.40	8.7	12.7	5.2	1.097	0.906	0.420	1.21
Soy beans	Peking	26	21	3.40	10.1	14.7	3.4	0.583	0.376	0.359	1.55
Sunflower	Mammoth Russian	22	25	4.56	15.0	14.0	3.9	1.250	0.757	0.607	1.65
Squash	Hubbard warted	5	33	5.10	6.3	17.0	4.5	4.124	3.032	2.610	1.36
Melon	Red Rocky Ford	30	33	5.62	5.2	6.1	1.3	0.371	0.303	0.221	1.22
Tomato	Bonnie Best	22	21	.....	2.4	7.5	0.5	0.060	0.044	0.038	1.36

agree with those reported by DEUBER (4) for soy bean seedlings. Some of the seedlings had traces of red color in the leaves. Definite changes in pigmentation developed during the growth of the corn seedlings. The leaves of the high-protein corn seedlings had no red color except traces in the midrib at the time of harvest. The low-protein corn seedlings, on the contrary, had much red in the leaves, were much less green, and especially were less bluish-green. The stems of both the high- and low-protein seedlings were very red. Although no red color developed in the wheat seedlings, there were similar differences in the amount and shade of green in the leaves of the high-protein (Marquis) and low-protein (Little Club) types.

2. *Seedlings grown with nitrates*

The data are presented in table IV. Most of the seedlings began to synthesize nitrates at an early stage of growth. This was especially true of seedlings of the grass family. The leguminous types did not use nitrates rapidly. The composition of the nutrient solution may not have been so well adapted for the growth of seedlings of this family as for the other kinds grown. Also these seedlings may be more sensitive to light conditions than some of the other types, and may require a greater amount of light to enable them to effect a rapid synthesis of nitrates into growth-promoting substances. In some other experiments performed during April and May, when light was of greater intensity and the days were longer, there was a much more rapid synthesis of nitrates by leguminous seedlings as indicated by growth. It may also be possible that, in the sterile cultures, the lack of nodules containing the nitrogen-fixing bacteria may have contributed somewhat in making these seedlings less efficient than the other types in the metabolism of nitrates.

Table V summarizes the effects of the utilization of nitrates on the growth of various organs. There is a striking difference in the effect of nitrates on the growth of shoots and roots. Nitrates increased growth of shoots remarkably, whereas eleven of the nineteen types grown had the total green weight of roots very little affected, and of these eleven types, five varied less than 5 per cent from the weights of roots of seedlings not receiving nitrates, and in four types the roots weighed less when nitrates were present than when they

TABLE IV

SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE WITH NITRATES, OCTOBER 7, 1925

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	TOTAL NITROGEN IN SEEDS (%)	AVERAGE LENGTH OF			GREEN WEIGHT PER PLANT			RATIO OF WEIGHTS OF SHOOTS TO ROOTS
					Shoots (cm.)	Roots (cm.)	Leaves (cm.)	Shoots (gm.)	Roots (gm.)	Leaves (gm.)	
Wheat	Blue stem	53	14	2.27	23.0	15.2	19.0	0.277	0.213	0.197	1.30
Wheat	Marquis	53	13	2.50	22.5	15.2	18.0	0.262	0.198	0.189	1.32
Wheat	Little club	56	13	1.52	19.7	14.5	13.4	0.205	0.197	0.148	1.04
Barley	Vermont champion	43	14	1.82	22.6	14.2	17.4	0.355	0.189	0.257	1.93
Oats	Storm king	46	18	2.68	28.0	9.2	21.5	0.405	0.274	0.262	1.47
Oats	Clydesdale	30	15	2.82	19.5	11.7	14.0	0.220	0.270	0.190	1.22
Rye	Giant winter	44	15	1.75	18.7	13.6	14.2	0.242	0.175	0.173	1.38
Rice	Honduras	44	22	1.68	8.1	6.3	4.7	0.060	0.060	0.026	1.01
Corn	Illinois high-protein	10	17	2.76	32.2	16.7	24.3	2.587	1.468	1.472	1.36
Corn	Illinois low-protein	10	17	1.09	26.8	17.0	19.4	1.946	1.547	1.049	1.25
Corn	Illinois high-oil	10	19	.....	31.6	16.5	24.0	2.512	2.559	1.597	0.98
Corn	Illinois low-oil	5	19	.....	30.4	14.2	23.2	2.776	2.582	1.618	1.07
Peas	Juno	18	33	5.10	20.0	14.2	3.0	2.730	1.815	.....	1.50
Peas	Canada white	16	23	3.60	21.6	13.2	2.5	1.348	0.897	.....	1.50
Beans	White Marrow bush	5	18	3.85	15.2	12.0	8.0	4.260	2.120	2.610	2.01
Cow peas	New era	15	17	4.40	11.0	13.0	6.0	1.658	1.008	0.978	1.62
Soy beans	Peking	26	21	3.40	11.3	11.4	3.6	0.791	0.449	0.513	1.76
Sunflower	Mammoth Russian	22	25	4.56	24.2	10.1	7.6	4.788	1.328	2.632	3.60
Squash	Hubbard warted	5	33	5.10	13.9	10.6	10.4	12.840	3.920	6.804	3.27
Melon	Red Rocky Ford	30	33	5.62	7.5	7.3	3.5	0.782	0.307	0.456	2.55
Tomato	Bonnie Best	24	21	.....	2.5	7.5	5.2	0.296	0.074	0.206	4.00

TABLE V

EFFECT OF UTILIZATION OF NITRATES UPON GROWTH OF SEEDLINGS KEPT IN NORMAL ATMOSPHERE

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	TOTAL NITROGEN IN SEEDS (%)	TOTAL WEIGHT OF SEEDS (GM.)	PERCENTAGE INCREASE IN GREEN WEIGHT DUE TO UTILIZATION OF NITRATES			
						Total	Shoots	Roots	Leaves
Wheat	Blue stem	53	14	2.27	2.05	+ 42.0	+ 89.0	+ 7.3	+ 94.9
Wheat	Marquis	53	13	2.50	1.84	+ 34.4	+ 83.8	− 0.8	+ 83.3
Wheat	Little club	56	13	1.52	1.86	+ 63.6	+112.9	+30.7	+176.3
Barley	Vermont champion	43	14	1.82	1.14	+ 66.8	+174.6	− 4.3	+158.1
Oats	Storm king	46	18	2.68	1.40	+ 54.5	+136.7	+ 2.2	+126.9
Oats	Clydesdale	30	15	2.82	1.35	+ 23.7	+ 90.1	+13.3	+ 95.9
Rye	Giant winter	44	15	1.75	1.01	+ 50.4	+182.0	− 8.5	+201.9
Rice	Honduras	44	22	1.68	1.04	− 3.8	+ 7.7	−13.2	+ 14.8
Corn	Illinois high-protein	10	17	2.76	2.73	+ 48.7	+ 75.7	+22.8	+ 75.1
Corn	Illinois low-protein	10	17	1.09	3.91	+143.5	+206.9	+93.4	+212.2
Corn	Illinois high-oil	10	19	.....	2.73	+ 64.2	+ 60.9	+44.1	+103.6
Corn	Illinois low-oil	5	19	.....	3.04	+ 50.8	+ 73.5	+32.2	+ 99.2
Peas	Juno	18	33	5.10	4.73	+ 24.4	+ 40.9	+ 5.7	.....
Peas	Canada white	16	23	3.60	2.33	+ 22.3	+ 42.3	+ 0.8	.....
Beans	White Marrow bush	5	18	3.85	1.66	+ 39.8	+ 41.1	+37.3	+ 46.5
Cow peas	New era	15	17	4.40	1.95	+ 32.1	+ 49.3	+11.2	+133.0
Soy beans	Peking	26	21	3.40	1.75	+ 29.6	+ 35.6	+19.4	+ 42.7
Sunflower	Mammoth Russian	22	25	4.56	1.04	+204.6	+282.9	+75.4	+333.1
Squash	Large Hubbard warted	5	33	5.10	0.73	+133.7	+211.4	+29.2	+160.6
Muskmelon	Red Rocky Ford	30	33	5.62	0.40	+ 61.5	+111.0	+ 1.3	+106.3
Tomato	Bonnie Best	22	21	.....	0.06	+255.7	+393.3	+68.2	+442.1

were not. It is true, however, that the roots of most of the seedlings receiving nitrates had somewhat more branching than those of the plants starved for nitrogen. The roots of most of the nitrated plants were shorter. The strengthening tissues of both roots and stems in most of the seedlings had somewhat less thickening of the cell walls, and the storage carbohydrates were in all cases less abundant than in the plants starved for nitrogen. The total increase due to the use of nitrates was greater for the high-protein, high-oil types of seedlings, but this was partially because these seedlings grew for a longer time. Of the three kinds of wheat grown, the starchy low-protein type (Little Club) made the greatest gain with the use of nitrates; and in the case of corn the starchy low-protein made a greater gain than the less starchy high-protein type.

The influence of light on the growth of different parts of seedlings is shown in table VI.

Effect of light on plants grown without extra nitrogen

The total green weight increased in plants grown without nitrogen, especially in the high-protein forms. The low-protein starchy types gained only slightly. The green weights of stem plus petioles all decreased under the influence of light, and the decreases ranged from 3.1 per cent in the case of stems of cow pea seedlings to 65.1 per cent in the case of stems of soy bean seedlings. There appeared to be no difference between the high- and low-protein types in the responses of stems and petioles to light. In all these experiments the leaf sheaths and petioles have been included with stem tissue, as they were considered to approach more nearly to stem than to leaf tissues in content and composition. The leaves and cotyledons gained in green weight in the light in most cases; however, the leaves of the starchy low-protein seedlings decreased. This loss in weight amounted to 37.8 per cent for Little Club wheat and 41.6 per cent for Illinois low-protein corn. In a general way it may be stated that the higher the nitrogen content of the seed, the greater the increase in green weight of leaves due to the influence of light. The leaves of leguminous seedlings were very responsive, and the growth of leaves of the high-protein, high-oil seedlings was remarkably influenced by light.

TABLE VI

PERCENTAGE INCREASE OR DECREASE IN WEIGHT OF VARIOUS ORGANS DUE TO INFLUENCE OF LIGHT, OCTOBER 1925

PLANT	VARIETY	PLANTS GROWN WITHOUT NITRATES					PLANTS GROWN WITH NITRATES AVAILABLE		
		Total	Shoots	Stems+ petioles	Leaves+ cotyledons	Roots	Total	Shoots	Roots
Wheat	Blue stem	+ 57.1	− 3.3%	−32.8%	+ 20.2%	+191.2	+ 95.2	+ 41.3%	+ 287.3
Wheat	Marquis	+ 55.0	−14.4	−51.2	+ 19.8	+268.5	+109.0	+ 51.4	+ 321.3
Wheat	Little club	+ 9.3	−39.2	−39.4	− 37.8	+125.8	+ 56.4	+ 13.8	+ 155.8
Barley	Vermont champion	+ 65.5	− 5.1	−53.2	+ 35.6	+219.3	+103.8	+ 88.8	+ 225.3
Oats	Storm king	+ 86.0	+14.0	−22.2	+ 47.4	+211.6	+211.4	+181.2	+ 270.2
Rye	Giant winter	+ 7.2	−23.9	−30.9	− 19.7	+ 75.0	+100.5	+ 56.1	+ 230.2
Rice	Honduras	− 0.8	−41.0	−56.0	+ 15.0	+122.6	− 49.1	− 48.7	+ 215.8
Corn	Illinois high-protein	+ 39.7	+ 0.1	−18.9	+ 20.4	+124.5	+129.4	+ 93.0	+ 243.8
Corn	Illinois low-protein	− 29.7	−50.8	−58.3	− 41.6	+ 6.6	+ 54.6	+ 11.2	+ 203.3
Corn	Illinois high-oil	+ 42.0	− 6.4	−15.1	+ 1.6	+129.9	.....	.....	.....
Corn	Illinois low-oil	+ 29.1	−11.1	−27.9	+ 15.0	+105.4	+187.9	+ 87.5	+ 584.8
Peas	Juno	+105.1	+52.6	.....	.....	+234.7	+176.4	+118.4	+ 360.6
Beans	White Marrow bush	+ 81.1	+51.4	−32.8	+1480.1	+193.5	+107.4	+ 64.2	+ 338.9
Cow peas	New era	+101.5	+44.9	− 3.1	+ 624.1	+282.3	+195.5	+128.6	+ 469.5
Soy beans	Peking	+ 17.8	+ 9.2	−65.1	+ 432.6	+118.6	+ 34.8	− 3.7	+ 358.2
Sunflower	Mammoth Russian	+ 78.4	+22.5	−36.9	+ 268.0	+620.9	+373.3	+305.7	+1085.7
Squash	Hubbard warted	+116.9	+43.8	−47.2	+ 401.9	+601.8	+206.4	+167.9	+ 479.6
Melon	Rocky Ford	+131.6	+47.8	−40.2	+ 449.7	+656.0	+264.2	+196.2	+ 777.1
Tomato	Bonnie Best	+131.1	+50.0	−45.0	+ 387.2	+780.0	+469.2	+385.2	+1750.0

One of the most noticeable effects of light is that of the strongly positive influence which it exerts on the growth of roots. The low-protein starchy types had this stimulating effect of light on growth of roots much less than all other kinds of seedlings. The roots of starchy low-protein corn seedlings gained 6.6 per cent by the action of light, the roots of rye 75 per cent, and Little Club wheat 125 per cent. Light caused roots of leguminous seedlings to gain from 119 to 282 per cent. The roots of the higher protein seedlings had much greater increases. The gains in weights of roots of high-protein, high-oil seedlings ranged from 602 to 780 per cent.

Effect of light on plants receiving nitrates

The total green weight was increased in the nitrated plants exposed to light as compared with those grown in darkness, with the exception of rice, in which there was no increase either in weight or in size. This feature of the behavior of rice has been pointed out previously. A peculiarity in the growth of the rice seedlings in darkness is that plants receiving and not receiving nitrates both had very much longer shoots than the corresponding plants grown in the light. Light caused an increase in growth of shoots (stems plus leaves) in most types of seedlings, and growth of roots was greatly increased in all of them. The gains in weights of roots ranged from 155 per cent for Little Club wheat to 1750 per cent for tomato seedlings.

EXPERIMENT II, MARCH 5–26, 1926

This experiment was conducted to determine the weekly increments of growth made by various types of seedlings when receiving and not receiving nitrate nitrogen. In the previous experiment it had been found that different seedlings required different lengths of time to use the reserve nitrogen of the seed, and since the seedlings receiving nitrates were harvested at the same time as seedlings starved for nitrogen, it was difficult to compare one type with another because of the different lengths of the period of growth. This experiment was planned to overcome this difficulty. This kind of an experiment, although better for studying the ability of various types of seedlings to utilize nitrates at different stages of germination and early growth, is not so good for comparing the ability of different types to grow

without extra nitrogen. When seedlings of the grass family were two weeks of age at this season of the year many of them had almost ceased growing, although they were still increasing slightly in weight. The higher protein types were still growing by the end of the third week and had not then attained maximum size.

1. Seedlings not receiving nitrates

The quantitative results are given in table VII. Illustrations of tomato and sunflower seedlings are shown in figs. 14, 16, 18, 20, 22, 24, 26, and 28. The weight of shoots in proportion to that of roots tended to decrease week by week. The relative proportion of shoots to roots of seedlings grown from starchy seeds diminished conspicuously during the second week, but there was relatively little change during the third week. The same situation was found with the higher protein seedlings, with the exception of squash, which grew relatively slowly during the first week. As in the previous experiment, the high-protein seedlings had higher shoot to root ratios than the low-protein seedlings. With the exception of the high-protein barley (Dickinson, N.D.), the greatest gains in green weight were made by the roots of all types of seedlings during the second week. Shoots of seedlings of the grass family and of sunflower grew most rapidly during the first week, and those of the leguminous type and squash and tomato during the second week.

2. Seedlings receiving nitrates

The quantitative results are shown in table VIII, and illustrations of tomato and sunflower seedlings in figs. 15, 17, 19, 21, 23, 25, 27, and 29. The shoot to root ratios were higher in the cultures receiving nitrates than in those not receiving them during the entire period of growth, although in several instances the differences at the end of the first week were not appreciable. The ratios of shoots to roots of the nitrated plants tended to become progressively higher week by week, whereas those of the nitrogen-limited plants became progressively lower. With the exception of seedlings of the legumes, there were marked increases in length of shoots and decreases in length of roots with the use of nitrates. Most of the nitrated plants had more branching of the roots, and the leaves were longer and wider.

TABLE VII

SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE AND HARVESTED AT END OF ONE, TWO, AND THREE WEEKS OF GROWTH, WITHOUT NITRATES, MARCH–APRIL, 1926

Plant	Variety	No. of plants	Total nitrogen in seeds (%)	Weight of seeds (gm.)	One week			Two weeks			Three weeks			Average length at end of third week			Green weight of tissue produced at end of third week per 0.1 gm. of reserve nitrogen		
					Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Shoots (cm.)	Roots (cm.)	Leaves (cm.)	Total (gm.)	Shoots (gm.)	Roots (gm.)
Wheat	Marquis	62	2.50	2.44	0.089	0.105	0.85	0.134	0.233	0.57	0.206	0.352	0.58	15.8	14.0	12.6	56.7	20.95	35.74
Wheat	Little club	62	1.52	1.87	0.062	0.103	0.60	0.085	0.219	0.39	0.133	0.322	0.41	11.2	13.9	8.8	99.6	29.15	70.42
Oats	Clydesdale	62	2.82	2.78	0.096	0.110	0.88	0.144	0.244	0.58	0.167	0.297	0.56	16.4	15.8	13.2	36.6	13.20	23.46
Barley	Hännchen (Dickinson)	62	2.76	2.11	0.155	0.247	0.62	0.205	0.340	0.60	0.267	0.381	0.70	21.5	21.5	15.2	69.0	28.43	40.58
Barley	Hännchen (Aberdeen)	62	2.20	2.50	0.138	0.156	0.88	0.185	0.345	0.53	0.201	0.376	0.53	17.6	17.6	12.6	65.0	22.65	42.38
Cow peas	New era	10	4.40	1.30	0.295	0.117	2.52	0.772	0.660	1.18	(−5 leaves 0.610)	0.985	0.62	6.2	11.3	4.0	27.9	(−5 leaves 10.66)	17.22
Peas	First of all	32	4.11	5.57	0.110	0.141	0.78	0.570	0.540	1.06	1.180	1.120	1.05	29.1	13.2	2.5	32.1	16.49	15.66
Soy bean	Peking	32	3.40	2.15	0.359	0.127	2.81	0.590	0.313	1.88	0.630	0.450	1.40	11.2	12.0	3.7	47.2	27.57	19.69
Squash	Hubbard	14	5.10	2.04	0.693	0.338	2.05	2.893	1.163	2.48	3.750	2.581	1.45	7.5	18.2	4.3	85.2	50.48	34.75
Sunflower	Mammoth Russian	20	4.56	0.95	0.635	0.257	2.47	0.943	0.575	1.64	1.210	0.976	1.24	13.9	11.3	3.4	100.9	55.89	45.08
Tomato	Bonnie Best	62	.....	0.16	0.030	0.013	2.28	0.070	0.046	1.51	0.075	0.073	1.02	2.7	10.1	1.2	125.6	63.5	62.1

TABLE VIII

SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE AND HARVESTED AT END OF ONE, TWO, AND THREE WEEKS OF GROWTH, WITH NITRATES, MARCH–APRIL, 1926

PLANT	VARIETY	NO. OF PLANTS	TOTAL NITROGEN IN SEEDS (%)	WEIGHT OF SEEDS (gm.)	ONE WEEK			TWO WEEKS			THREE WEEKS			AVERAGE LENGTH AT END OF THIRD WEEK			GREEN WEIGHT OF TISSUE PRODUCED AT END OF THIRD WEEK PER 0.1 GM. OF RESERVE NITROGEN		
					Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Shoots (cm.)	Roots (cm.)	Leaves (cm.)	Total (gm.)	Shoots (gm.)	Roots (gm.)
Wheat	Marquis	62	2.50	2.44	0.150	0.113	1.32	0.408	0.279	1.46	0.893	0.328	2.72	36.6	8.3	27.8	124.1	90.81	33.3
Wheat	Little club	62	1.52	1.87	0.097	0.115	0.84	0.277	0.239	1.15	0.668	0.331	2.01	31.6	11.2	25.2	218.1	145.8	72.3
Oats	Clydesdale	62	2.82	2.78	0.149	0.150	0.99	0.394	0.269	1.47	0.871	0.318	2.73	42.9	13.9	29.1	94.1	68.9	25.2
Barley	Hännchen (Dickinson)	62	2.76	2.11	0.210	0.212	0.99	0.665	0.362	1.83	1.120	0.447	2.51	37.9	10.7	30.4	166.9	119.3	47.6
Barley	Hännchen (Aberdeen)	62	2.20	2.50	0.233	0.261	0.89	0.731	0.379	1.92	1.110	0.433	2.57	37.8	9.2	30.3	173.9	125.1	48.8
Cow peas	New era	10	4.40	1.30	0.332	0.130	2.55	1.339	0.804	1.66	1.390	1.009	1.37	5.0	8.2	5.0	41.9	24.3	17.6
Peas	First of all	32	4.11	5.57	0.123	0.134	0.92	0.915	0.656	1.39	1.353	0.681	1.98	29.1	5.0	2.5	28.4	18.9	9.5
Soy bean	Peking	32	3.40	2.15	0.360	0.106	3.41	0.702	0.300	2.33	0.880	0.518	1.70	13.9	10.0	4.4	61.2	38.5	22.7
Squash	Hubbard	14	5.10	2.04	1.579	0.468	3.37	6.107	1.736	3.52	14.500	3.403	4.26	35.4	17.0	10.0	241.0	195.2	45.8
Sunflower	Mammoth Russian	20	4.56	0.95	0.714	0.245	2.91	2.011	0.611	3.29	4.660	0.901	5.16	32.8	11.3	8.2	72.2	55.6	16.5
Tomato	Bonnie Best	62	.....	0.16	0.067	0.015	4.40	0.434	0.061	7.15	1.135	0.134	8.46	20.2	8.1	7.5	256.8	215.2	41.6

Both shoots and roots of all the types of seedlings made the greatest gains in green weight during the second week. At the end of the first week the nitrated seedlings of the grass family had greener leaves than the seedlings not receiving nitrogen. Oat seedlings were an exception in this respect. There was no difference in the greenness of the two sets of leaves in the three types of leguminous seedlings. This condition was correlated with little difference in size of the nitrated and un-nitrated cultures. There was very little difference in color of the cotyledons of tomato, squash, and sunflower seedlings of the two sets of cultures at the end of the first week.

At the end of the second week the first leaves of seedlings of the grass family which were not receiving nitrates were beginning to die at the tips, and the general color of the leaves was a yellower green than that of the nitrated seedlings. With the exception of cow pea seedlings, in which there was no difference in color, the leguminous seedlings not receiving nitrates had greener leaves at the end of the second week. The cotyledons and leaves of sunflower and squash and the cotyledons of tomato seedlings not receiving nitrates were greener than those of plants receiving nitrates.

At the end of the third week all plants receiving nitrates had greener leaves than those of the nitrogen-starved plants.

It has been found in both the March (1925) and October (1926) experiments that seedlings of the grass family developed signs of starvation for nitrogen earlier than the higher protein types. Seedlings from the latter type of seeds had the capacity to continue growth for a longer time. There also appeared to be a difference in the amount of green tissue produced per 0.1 gm. of reserve nitrogen. The results of the October experiment are given in table IX, and of the March experiment in tables X and XI. The four types of high-protein, high-oil seedlings differ from all the others in this respect. Per unit of reserve nitrogen their total green weight is greater, and the difference in weight as compared with seedlings grown from the low-protein seeds is brought about by a greater growth of stems and leaves. There is no distinct difference in the weight of roots produced per unit of nitrogen by the different classes of seedlings. Just what is the cause or significance of this increased growth by the high-protein, high-oil seedlings cannot be stated. The high-protein, high-oil types

TABLE IX

GREEN WEIGHT OF TISSUE IN GRAMS PRODUCED PER 0.1 GM. OF RESERVE NITROGEN BY SEEDLINGS GROWN WITH NO EXTERNAL SOURCE OF NITROGEN, MARCH–APRIL, 1924

PLANT	VARIETY	AMOUNT OF N PER SEED (MG.)	DARKNESS					LIGHT				
			Total	Shoots (stems + leaves)	Stems + petioles	Leaves + cotyledons	Roots	Total	Shoots (stems + leaves)	Stems + petioles	Leaves + cotyledons	Roots
Wheat	Blue stem	0.878	24.9	17.2	7.8	9.4	7.7	35.4	15.0	4.62	10.4	20.4
Wheat	Marquis	0.982	22.4	16.9	8.2	8.7	5.5	32.4	13.5	3.73	9.76	18.9
Wheat	Little club	0.504	44.4	31.3	14.1	17.2	13.1	48.6	19.0	8.53	10.5	29.6
Barley	Vermont champion	0.482	40.9	28.0	12.9	15.1	12.8	62.0	24.4	5.48	18.9	37.6
Oats	Storm king	0.817	28.8	18.3	8.8	9.5	10.5	49.1	19.1	6.21	12.9	30.0
Rye	Giant winter	0.401	41.1	28.1	10.4	17.7	12.9	61.9	19.2	6.39	12.8	42.7
Rice	Honduras	0.396	31.7	24.0	19.0	5.0	7.8	29.0	13.0	7.67	5.34	16.0
Corn	Illinois high-protein	7.53	28.6	19.5	10.3	9.2	9.1	40.0	19.5	8.42	11.1	20.4
Corn	Illinois low-protein	4.26	47.8	30.2	16.7	13.5	17.6	33.7	14.9	6.99	7.88	18.8
Peas	Juno	13.4	13.3	9.4	8.5	0.9	3.8	25.6	13.6	6.29	7.30	12.0
Beans	White Marrow bush	12.8	19.7	15.5	14.6	0.9	4.1	32.3	21.4	8.95	12.4	10.9
Cow peas	New era	5.74	17.2	13.0	12.9	0.1	4.1	26.8	14.7	9.05	5.61	12.1
Soy beans	Peking	2.29	35.5	28.0	25.1	2.9	7.5	39.6	24.1	9.25	14.7	15.5
Sunflower	Mammoth Russian	2.17	51.8	47.0	39.4	7.6	4.8	80.3	50.0	25.7	24.3	30.3
Squash	Hubbard	9.83	33.5	29.1	23.8	5.3	4.4	67.9	39.1	14.7	24.8	28.8
Melon	Red Rocky Ford	0.753	38.6	33.3	28.0	5.3	5.3	83.4	45.9	18.6	27.3	37.5
Tomato	Bonnie Best	0.117	38.4	34.2	27.6	6.6	4.2	80.0	51.2	18.7	32.5	37.6

TABLE X

GREEN WEIGHT OF TISSUES IN GRAMS PRODUCED PER 0.1 GM. OF RESERVE NITROGEN BY SEEDLINGS HAVING NO EXTERNAL SOURCE OF NITROGEN, MARCH 5–26, 1926

PLANT	VARIETY	NO. OF PLANTS	TOTAL NITROGEN IN SEEDS (%)	WEIGHT OF SEEDS (GM.)	ONE WEEK			TWO WEEKS			THREE WEEKS		
					Total	Shoots	Roots	Total	Shoots	Roots	Total	Shoots	Roots
Wheat	Marquis	62	2.50	2.44	20.50	9.83	10.72	37.40	13.67	23.70	56.70	20.95	35.74
Wheat	Little club	62	1.52	1.87	32.40	12.17	20.22	59.82	16.80	43.07	89.70	26.24	63.38
Oats	Clydesdale	62	2.82	2.78	16.30	7.59	8.67	30.7	11.40	19.28	36.6	13.20	23.46
Barley	Hännchen (Dickinson)	62	2.76	2.11	42.80	16.48	26.32	58.10	21.84	36.22	69.00	28.43	40.58
Barley	Hännchen (Aberdeen)	62	2.20	2.50	33.10	15.56	17.58	59.70	20.85	38.89	65.00	22.65	42.38
Cow peas	New era	10	4.40	1.30	7.20	5.16	2.04	25.00	13.49	11.54	27.90	5 leaves had dropped off 10.66	17.22
Peas	First of all	32	4.11	5.57	3.50	1.54	1.96	15.5	7.97	7.55	32.1	16.49	15.66
Soy bean	Peking	32	3.40	2.15	21.3	15.7	5.58	39.5	25.83	13.71	47.2	27.57	19.69
Squash	Hubbard	14	5.10	2.04	13.9	9.33	4.55	54.6	38.94	15.65	85.2	50.48	34.75
Sunflower	Mammoth Russian	20	4.56	0.95	41.2	29.33	11.87	70.1	43.57	26.56	100.9	55.89	45.08
Tomato	Bonnie Best	62	0.00729 gm. in 62 seeds	.....	36.8	25.6	11.2	99.0	59.5	39.5	125.6	63.5	62.1

TABLE XI

GREEN WEIGHT OF TISSUES IN GRAMS PRODUCED PER 0.1 GM. OF RESERVE NITROGEN BY SEEDLINGS RECEIVING NITRATES, MARCH 5–26, 1926

PLANT	VARIETY	NO. OF PLANTS	TOTAL NITROGEN IN SEEDS (%)	WEIGHT OF SEEDS (GM.)	ONE WEEK			TWO WEEKS			THREE WEEKS		
					Total	Shoots	Roots	Total	Shoots	Roots	Total	Shoots	Roots
Wheat	Marquis	62	2.50	2.44	26.82	15.28	11.54	69.92	41.54	28.38	124.1	90.81	33.34
Wheat	Little club	62	1.52	1.87	46.26	21.12	25.14	112.5	60.38	52.18	218.1	145.8	72.35
Oats	Clydesdale	62	2.82	2.78	23.71	11.83	11.88	52.43	31.17	21.26	94.10	68.87	25.19
Barley	Hännchen (Dickinson)	62	2.76	2.11	44.91	22.37	22.54	109.5	70.84	38.66	166.9	119.3	47.61
Barley	Hännchen (Aberdeen)	62	2.20	2.50	55.67	26.25	29.42	125.1	82.40	42.70	173.9	125.1	48.80
Cow peas	New era	10	4.40	1.30	8.07	5.80	2.27	37.46	23.41	14.05	41.90	24.30	17.64
Peas	First of all	32	4.11	5.57	3.59	1.72	1.87	21.95	12.79	9.16	28.40	18.91	9.51
Soy bean	Peking	32	3.40	2.15	20.37	15.75	4.62	43.89	30.72	13.17	61.20	38.52	22.66
Squash	Hubbard	14	5.10	2.04	27.55	21.25	6.30	105.6	82.21	23.36	241.0	195.2	45.82
Sunflower	Mammoth Russian	20	4.56	0.95	44.28	32.97	11.31	121.1	92.88	28.22	256.8	215.2	41.61
Tomato	Bonnie Best	62	0.00729 gm. in 62 seeds	.....	70.0	57.2	12.8	423.0	370.9	52.1	1084.5	970.0	114.5

of seedlings also have much the greatest efficiency in the metabolism of inorganic nitrogen.

Discussion

The questions raised in the introduction will now be considered.

1. What are the responses of seedlings having different amounts of reserve carbon and nitrogen when extra amounts of carbon and nitrogen are supplied externally?

The relations of varying amounts of available carbon and nitrogen to the growth of shoots and roots of seedlings are much like those found with tomato cuttings. Seedlings grown from seeds having large carbon reserves in proportion to the nitrogen tend to have low shoot to root ratios when grown without an external source of nitrogen, and those grown from seeds having relatively large amounts of nitrogen have higher shoot to root ratios if the seedlings are grown in the light. If the seedlings are grown in darkness there appears to be a somewhat similar, although considerably less definite, relation between the type of growth and the kind and amount of the food reserves.

The quantitative differences in the growth of different organs of seedlings kept in the light appear to be more directly related to the amounts of available carbon and nitrogen than to genetical affiliations. Within the grass family, for example, we find variations in growth related to the amount of stored foods. The very starchy, low-protein types of wheat, barley, and corn have lower shoot to root ratios than the corresponding somewhat less starchy and higher protein types. The results obtained with the four kinds of seedlings grown from high-protein, high-oil seeds, representing three different families, also indicate that the responses are related to the types and quantities of reserve foods.

When extra nitrogen is supplied, growth is in most cases slightly increased in darkness and is greatly increased in the light. The extra nitrogen favors growth of shoots more than that of roots. The roots of the nitrated seedlings are shorter. In no case was the increase with the use of nitrates in darkness equal to that obtained with the very high-carbohydrate tomato cuttings. It is supposed that this quantitative difference in the responses of seedlings as

compared with the cuttings resulted from the smaller amount of carbohydrate in proportion to the stored nitrogen in the seeds. Tomato stem cuttings may be produced which contain thirty-six parts of carbohydrates (starch, sugar, free-reducing substances) to one of nitrogen; whereas the starchy wheat grains have only about seven parts of carbohydrates to one of nitrogen. The carbohydrates may thus tend to become limiting factors in the growth of seedlings in darkness more quickly than they did in the growth of cuttings.

2. Is fertilization with nitrogen advisable in the early growth of all types of seedlings, regardless of weather and light conditions and the nitrogen reserves of the seed?

The results obtained in some experiments with seedlings grown at different seasons of the year show that, with the exception of rice seedlings, all the types can assimilate nitrate nitrogen into growth-promoting substances in the early phases of growth, if light conditions are favorable and permit of rapid and abundant synthesis of carbohydrates. On the other hand, if conditions are unfavorable for the synthesis of carbohydrates, the very high-nitrogen types of seedlings may grow as well without as with nitrates in their early growth. Nitrates become beneficial after the seedlings have developed a photosynthesizing surface. The rate of nitrogen assimilation is closely correlated with the rate of synthesis of carbon compounds. This sort of response also agrees to some extent with results found with tomato cuttings. There it was noted that nitrates were toxic to cuttings having a very high-nitrogen but low-carbohydrate content when kept in darkness, and that nitrates were of no directly noticeable benefit in the light until more carbohydrates had accumulated by photosynthesis.

3. Is the seedling with the larger reserve of carbon better able to live and thrive during prolonged periods of dull, cloudy weather during the early growth of the plant?

4. Is the seedling with the larger amount of stored nitrogen at its disposal better able to withstand conditions of drought or of poor soil during the early phase of development when nitrogen from an outside source is unavailable or available in very limited amounts?

There is some evidence that those seedlings endowed by the parent plant with an abundance of readily available carbon com-

pounds will make the most rapid growth at first. This is true, not only of those with a limited nitrogen supply, but also of those grown from higher-nitrogen, high-fat seeds in which there is a great abundance of sugar produced by a rapid hydrolysis of the fats. Sunflower and tomato seedlings have this mode of behavior. These two types of seedlings and those of the grass family grew very rapidly during the first week. The results suggest that seedlings having an abundance of readily obtainable carbon reserves are better able to grow during cloudy weather in the early stages of growth. On the other hand, the seedlings having the larger stores of nitrogen are better able to grow and establish themselves in nitrogen-poor soil. They will tend to become deep-rooted under such conditions, and should be able to absorb a greater amount of nitrogen from the substrate because of the wider spread of roots. A period of abundant nitrogen and relatively small carbon supply in the early stages of growth tends to make leafy but shallow-rooted plants, which may not be so well adapted to obtain their needed amount of nitrogen and other minerals in their later development.

KOSINSKI (7), who was probably the first to study this relation of nitrogen to the growth of different organs, found that nitrogen feeding restricts the growth of roots in length and favors that of the stem. GODLEWSKI, in whose laboratory part of KOSINSKI's experiments were conducted (the work was begun in the botanical laboratory at Jena), incidentally utilized some of his barley seedlings for proof of KOSINSKI's results. His observations (6) agreed with those of KOSINSKI, and showed besides that the presence of sugar favored the growth of roots in length. He states that:

> root growth was especially favored in comparison with shoot growth if the nitrogen-free solution contained sugar, probably because in the more abundant supply of carbohydrate material, the nitrogen-hunger was accentuated. It seems as if the plants, hungering for nitrogen, strive through the appropriation of the largest possible quantity of the materials available for growth of roots to make better use of the scanty nitrogen supply in the soil.

In the same manner as GODLEWSKI has attempted to account for the favoring of growth of roots by a limited supply of nitrogen and an abundance of carbohydrates, one might explain the stimulation in growth of shoots (particularly of leaves that results when

nitrogen is abundant) as an effort on the part of the plant to expose a great amount of surface to the light so as better to provide for an increase in the products of photosynthesis. It seems more probable, however, that the differences in growth are due more directly to differences in the chemical conditions within the tissues rather than that they develop as purposeful adaptations.

GODLEWSKI (5) observed that:

> the roots of etiolated plants are usually shorter than those of plants grown in the light, yet the difference is so small that on no account can the greater length of the hypocotyl of (plants grown in darkness) be compensated by the decrease in length of the roots.

The effect of light on the growth of roots has been neglected by most of the investigators who have studied the characteristics of the etiolated plant in contrast with those of the plant grown in the light. Perhaps the most conspicuous feature of the results reported here is that of the stimulating effect of light on root development. The light-favoring effect on growth of roots is most pronounced in the case of seedlings grown from high-protein seeds. Some of the low-protein types, as low-protein corn, produce root systems as large in darkness as in light when nitrogen is lacking in the nutrient medium. A similar response was noted with tomato cuttings. If they had a very large supply of carbohydrates and were not given extra nitrogen, roots grew almost as well in darkness as in light. It thus seems probable that the light-favoring effect on the growth of roots is directly or indirectly connected with the synthesis of carbohydrates.

These experiments have also demonstrated the well known fact that light has a limiting influence on growth in length of stems. Whether the growth-inhibiting effect is due indirectly to the synthesis and accumulation of carbohydrates, or to its effect on nitrogen and other forms of mineral metabolism, or to more directly stimulating effects on the protoplasm itself, has long been a much discussed question, and one for which a definite answer is still lacking. If seedlings having a supply of reserve carbohydrates are grown in light in an atmosphere lacking carbon dioxide, growth of the stem may equal that of a plant kept in the normal atmosphere under conditions that permit the rapid synthesis of carbohydrates. From this it has been concluded that the growth-limiting effects of light

on the stem cannot be directly due to the synthesis of carbohydrates. GODLEWSKI'S (5) experiments have shown that a greater quantity of dry matter may pass from the cotyledons into the hypocotyl, if seedlings are grown in darkness than if grown in light in an atmosphere lacking carbon dioxide. He found that stems of 14-day old *Phaseolus* seedlings grown from seeds of the same weight contained 202 mg. of dry matter when grown in darkness, and 141 mg. of dry matter when grown in light in air lacking CO_2. It may be concluded that the hypocotyls of etiolated plants are longer than those of plants grown in the light, partly because more of the food reserves are used in their growth, and also because they have a considerably higher water content.

GODLEWSKI (5) also sought to determine whether the growth-limiting effects of light on the hypocotyl were exerted directly on it, or more indirectly through its influence in favoring the growth of the cotyledons. Using black paper cases, he darkened the cotyledons only of some etiolated *Raphanus* seedlings and the hypocotyls only in others. Similar seedlings were left uncovered and set out in normal atmosphere. He found that darkening the hypocotyl only caused a definite increase in its length; secondary to this, the cotyledons of the plants with the darkened hypocotyls were somewhat smaller than those of plants grown wholly in the light. Darkening the cotyledons only had a strong influence on their growth; they were larger than the cotyledons of completely etiolated plants. He stated, however, that the cotyledons became somewhat green at the base. It may be possible that the traces of light, which undoubtedly must have entered, caused a stimulation in growth of the cotyledons. BATALIN (1) presented some experimental evidence which showed that illumination of short duration exerts a remarkable influence on the growth of leaves. GODLEWSKI also has shown that illumination for a brief period favors the growth of cotyledons of *Raphanus* seedlings. Later experiments of TRUMPF (15) have demonstrated similar results.

The effects of light on the growth and development of different organs of seedlings will be discussed more fully in two following papers. Additional data will also be presented which help somewhat to indicate to what extent the influence of light on the growth of

different organs is due to the processes of assimilation and utilization of carbohydrates, and to what extent to the influence of light on metabolism and growth independently of carbohydrate synthesis.

Summary of results obtained with seedlings grown in light

1. Seedlings developed from seeds with high-nitrogen content, when grown with no external source of nitrogen, have a greater weight of shoots in proportion to that of roots than those which develop from seeds with low-nitrogen content.

2. The weights of shoots in proportion to roots of seedlings grown in the light is lower than those of seedlings grown in darkness. The shift in the proportions is brought about especially by the shorter stems, and in most cases by much larger root systems of the seedlings grown in the light.

3. The difference in the effect of light and darkness upon the weight of tops in proportion to roots is greater with seedlings from high-protein seeds grown without extra nitrogen than with seedlings grown from low-protein seeds.

4. Light does not greatly favor growth of seedlings from low-protein starchy seeds unless extra nitrogen is supplied. The leaves of these plants are considerably smaller and even weigh less in the light. The roots of some of these seedlings gain considerably in the light, however.

5. Light favors the assimilation of nitrates, especially by high-protein seedlings with relatively low carbon reserves. The assimilation of nitrates and utilization of the synthesized products favor the growth of shoots more than of roots.

6. When the nitrogen supply is limited to the reserves in the seed, the shoot to root ratios tend to diminish after the first week, but if nitrogen is abundantly supplied, the ratio tends to increase continuously during the first three weeks of growth of seedlings in the light.

7. The relative proportions of shoots to roots vary with the season of the year. In the long-day months the ratio is relatively low and in the short-day months it is higher.

8. Light favors the process of secondary thickening in the roots

and stems of dicotyledons, and of thickening of cell walls in all types of seedlings.

General summary

1. Growth of the seedling is influenced by the nature and relative amounts of the food reserves of the seed, as well as by differences in the external environment such as light and darkness, and the presence and absence of nitrates in the nutrient solution. When the seedlings are grown without nitrogen from an outside source the following responses have been found: (a) Seeds having a high-nitrogen and relatively low-carbon content produce seedlings with a large top in proportion to the roots. (b) Seeds having a low-nitrogen and high-carbon content produce seedlings with a relatively small top in proportion to the size and weight of the roots. (c) Seeds intermediate in the proportions of their reserves of carbon and nitrogen produce seedlings with intermediate proportions of shoots to roots.

The following table illustrates the nature of the results:

TABLE XII

WEIGHT IN GRAMS OF SHOOTS AND ROOTS PER PLANT

	Reserve foods	Light			Darkness		
		Stem petioles	Leaves	Roots	Stems	Leaves	Roots
High-protein corn	Moderately high nitrogen / Moderately high starch	0.634	0.838	1.540	0.774	0.696	0.686
Low-protein corn	Low nitrogen / High starch	0.298	0.336	0.800	0.715	0.575	0.750
White Marrow beans	Moderately high nitrogen / Moderately high starch	1.264	1.754	1.544	1.882	0.111	0.526
Cow peas	High nitrogen / Moderately low starch	0.677	0.420	0.906	0.699	0.058	0.237
Sunflower	High nitrogen / High oil	0.643	0.607	0.757	0.855	0.165	0.105
Muskmelon	Very high nitrogen / High oil	0.150	0.221	0.303	0.211	0.040	0.040

2. Nitrates are synthesized into growth-promoting substances, both in light and in darkness, but much more rapidly in the light.

3. Nitrates favor the growth of shoots more than of roots.

4. Light strongly favors the growth of roots.

5. (a) Seedlings developed from high-protein seeds benefit most under the influence of light. The roots and leaves are larger, more numerous, and much heavier than in the case of seedlings grown in darkness. This applies to high-protein seedlings grown with and without extra nitrogen, but the effect is greater in the case of the latter. (b) Seedlings grown from low-protein seeds without extra nitrogen are influenced less by light as to weight of different organs. Leaves of the very low-protein types grow even less in light than in darkness. When extra nitrogen is supplied these seedlings also benefit by the influence of light.

6. Seedlings with limited nitrogen supply undergo rapid differentiation and maturing of tissues in the light. The lower the nitrogen content of the seed the more rapid the process.

7. Light favors secondary thickening in stems and roots and deposition of strengthening materials in the cell walls.

8. The responses as to the effect of varying amounts of reserve carbon and nitrogen on growth of the seedling agree with results obtained with tomato cuttings having similar (although in some cases more extreme) variations in composition of the reserves.

These investigations were conducted at Boyce Thompson Institute for Plant Research, in 1924–1926. I wish to express my appreciation to the Cereals Division of the United States Department of Agriculture for the samples of pure line Marquis wheat and Hännchen barley used in these experiments; also to the Department of Plant Genetics of the University of Illinois for the seeds of high- and low-protein corn.

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EXPLANATION OF PLATES I–IV

PLATE I

Seedlings grown in darkness without nitrates:

FIG. 1.—Soy bean.
FIG. 2.—Cow pea.
FIG. 3.—Muskmelon.
FIG. 4.—Tomato.
FIG. 5.—Sunflower.
FIG. 6.—Illinois low-protein corn.
FIG. 7.—Illinois high-protein corn.

PLATE II

Seedlings grown in light without nitrates:

FIG. 8.—Soy bean.
FIG. 9.—Cow pea.
FIG. 10.—Muskmelon.

FIG. 11.—Sunflower.

FIG. 12.—Illinois low-protein corn.

FIG. 13.—Illinois high-protein corn.

PLATE III

Tomato seedlings grown in light:

FIGS. 14, 16, 18, and 20.—In nutrient medium lacking nitrogen at stages of growth at one, two, three, and four weeks respectively.

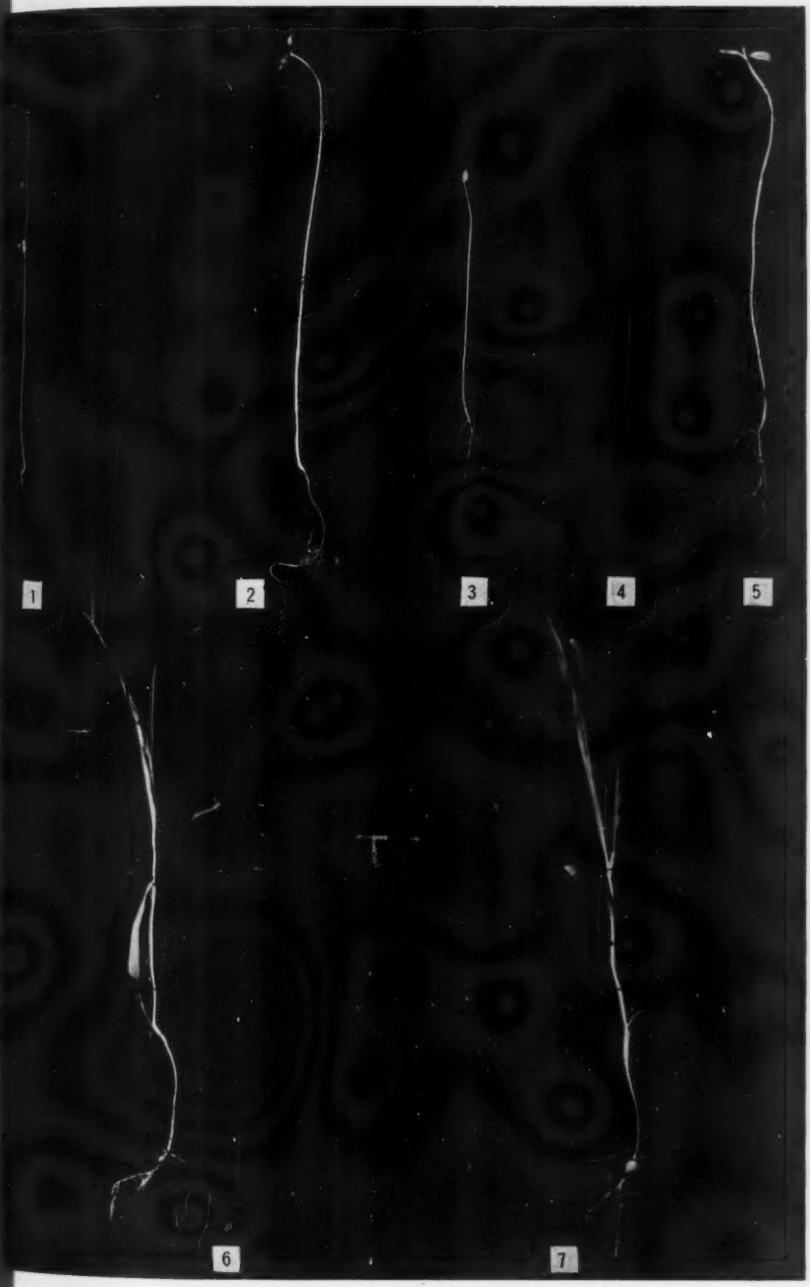
FIGS. 15, 17, 19, and 21.—In nutrient medium containing nitrates at stages of growth of one, two, three, and four weeks respectively.

PLATE IV

Sunflower seedlings grown in light:

FIGS. 22, 24, 26, and 28.—In nutrient medium lacking nitrogen at stages of growth of one, two, three, and four weeks respectively.

FIGS. 23, 25, 27, and 29.—In nutrient medium containing nitrates at stages of growth of one, two, three, and four weeks respectively.



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ORIGIN OF ADVENTITIOUS ROOTS IN COLEUS CUTTINGS[1]

MARGERY C. CARLSON

(WITH PLATES V, VI)

Introduction

In connection with some microchemical work on the rooting of *Coleus* cuttings, it was necessary to know the origin of their adventitious roots. The extensive work of LEMAIRE (1) was concerned with the origin of naturally occurring endogenous adventitious roots in the hypocotyls, stolons, and rhizomes of many herbaceous dicotyledons. He grouped the plants studied into the following four classes, of which the first is most common: (1) all tissues of the root originating in the pericycle of the stem; (2) the central cylinder of the root coming from the pericycle, and the other regions from the endodermis and inner cortex of the stem; (3) all tissues of the root coming from the "subphloem meristem" (cambium); (4) the central cylinder of the root formed by the cambium, and the other tissues by the pericycle of the stem.

VAN TIEGHEM and DOULIOT (5) added to their investigation of the origin of secondary roots a reinvestigation of the problem of the origin of natural adventitious roots in stems, chiefly hypocotyls and rhizomes. They came to the conclusion that endogenous roots arise entirely from the pericycle, except in older portions of stems where the pericycle has lost its "root-forming character." They define the pericycle as the layer, or layers, between the endodermis and the external phloem of a fibrovascular bundle, continuous across the medullary rays, but not distinguished on the inside from the medullary ray.

If the pericycle is simple, an arc of cells elongates radially and divides by tangential walls. The internal layer becomes the central cylinder of the new root. A tangential division of the external layer

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

separates the cortex (internal) from the epidermis (external). The cells of the epidermal layer surrounding the tip of the root divide again to form the root cap. If the pericycle is compound, it is usually its external layer which produces the epidermis, cortex, and central cylinder of the root. The other layers produce only the internal region of the central cylinder. When the root arises after the pericycle has lost its "root-forming" property, it may originate in the external primary phloem parenchyma, the internal primary phloem parenchyma, or the secondary phloem parenchyma.

Some recent work has dealt with the origin of adventitious roots in cuttings. SMITH (2), using cuttings of *Coleus blumei*, reports that "the first recognizable sign of the development of an adventitious root is the appearance in the cambium of a nest of highly meristematic cells." VAN DER LEK (4) described "root germs," present in young branches of *Ribes nigrum*, *Salix*, and *Populus*, as being "in connection with the cambium" as a "continuation of a medullary ray." SWINGLE (3) finds "root germs" initiated in the cambium ring of apple stems which produce burrknots.

Materials and methods

The work here reported was restricted to a study of the origin and development of adventitious roots arising between the fibrovascular bundles in young stem cuttings of *Coleus blumei*.

The cuttings, 2–6 inches long, were made from the tips of branches of *Coleus* plants and placed with their cut ends in water or in sand. The cuts were usually made through the internodes. The basal portions of these cuttings were removed and preserved at intervals beginning with the third day. These pieces of stems were fixed in formalin-acetic-alcohol and imbedded in paraffin. Sections, both transverse and longitudinal, were cut 12–15 μ in thickness and stained with safranin and gentian violet. In all cases serial sections were studied, and the median section of the primordium or young root was used for the figures.

In this paper the terms "origin" or "initial" will be used to indicate the cell, or group of cells, which by division initiate an adventitious root; "root primordium" to mean the group of meristematic cells from the time of earliest divisions of the "origin" to the

time of differentiation into well marked regions characterizing a young root.

Observations

The structure of the stem of *C. blumei* has been described by SMITH (2). The arrangement and differentiation of the tissues in the stems used in this work are shown in figs. 1, 3, and 5. The outer cortex at the stages shown has not yet developed into collenchyma. The endodermis is a somewhat regular layer of cells, usually smaller than the typical cortical parenchyma cells, and is easily detected because of the presence of large, compound starch grains (fig. 4). The pericycle consists of a single layer of cells just inside the endodermis. In stems older than those used for this study the pericycle opposite the vascular bundles differentiates into sclerenchyma.

Four large fibrovascular bundles occupy the angles of the stem. One or two smaller bundles lie between each two contiguous corner bundles (fig. 7). Isolated groups of primary phloem cells occur between the bundles. Such groups are seen in figs. 1 and 3. The layer of pericycle cells lies just outside these groups of cells.

A continuous cylinder of cambium was well developed in all the stems studied. In some cases the cambial cells had just begun to divide to form secondary xylem and phloem (fig. 7), but more commonly three to five layers of secondary xylem and one or two layers of secondary phloem were already present (figs. 1, 3, and 5). The interfascicular cambium arises one or two layers inside of the pericycle. There are, therefore, one or two layers of parenchymatous cells between the pericycle and the interfascicular cambium.

SMITH (2) finds that "the first roots appear in four ranks, corresponding to the four vascular strands. This arrangement may be obscured later by roots which arise irregularly in between." As a rule, the findings reported in this paper are in agreement with SMITH's observations.

Table I shows the age and position of root primordia and adventitious roots in seven cuttings chosen at random. The roots are numbered in the order of their appearance. The oldest root, that is, the first to appear, is numbered 1; the next younger, 2, etc. The numbers are arranged in columns with respect to the position of the roots in the stem from the cut surface upward; the lowest number

in a column therefore represents the lowest root in the stem, and so on. The letters following the numbers indicate the position of the adventitious roots with respect to the fibrovascular bundles. "C" indicates that the root appears opposite a large corner bundle; "EC," at the edge of a corner bundle; "L," opposite a lateral bundle; "B," between two bundles.

TABLE I

ORDER OF APPEARANCE AND POSITION OF ROOTS AND ROOT PRIMORDIA IN SEVEN COLEUS CUTTINGS

(EXPLANATION IN TEXT)

CUTTING NUMBER						
I	II	III	IV	V	VI	VII
1–C	1–C	3–EC	1–C	1–C	1–B	3–C
2–C	5–C	2–EC	2–C	5–EC	6–L	5–B
3–L	3–B	1–C	3–C	2–B	6–B	2–EC
4–C	2–C	4–EC	1–C	3–C	7–L	3–C
6–EC	4–C	1–C	4–B	4–C	2–C	1–C
5–C					7–L	3–C
					2–C	6–B
					4–C	7–B
					8–B	6–B
					5–EC	5–B
					3–C	4–EC
						5–B
						10–B
						6–B
						10–B
						6–B
						10–B
						7–B
						8–B
						9–B
						10–B

In general, the first four or five roots arose opposite corner bundles, while later roots arose opposite lateral bundles or between them. An exception was found in four of the cuttings examined. In cutting VI the first root arose between the bundles; in cutting V, the second; in cutting III, the third; and in cutting IV, the fourth.

There was no regularity in the appearance of the roots with respect to the cut surface. Sometimes the first roots arose nearest the cut surface (table I, cuttings III and IV), and sometimes farthest from the cut surface (cuttings I, II, IV, V, VI, and VII). All roots appeared within a distance of 2–3 mm. from the base of the cuttings.

The first evidence of adventitious root formation is an accumulation of protoplasm and an increase in the size of the nucleus and of the nucleole in one cell or in several adjacent cells of the pericycle. The enlarged nucleus takes a central position. The cell or cells then divide.

Fig. 1 shows three neighboring pericyclic cells which have divided, thus initiating an adventitious root. The cell on the left divided tangentially, and then the inner daughter cell divided tangentially. The middle cell of the group divided by a tangential wall; its inner daughter cell divided by a tangential wall and then the outer granddaughter cell divided slightly obliquely. The first division of the cell on the right of the group was also tangential.

In fig. 2 a pericyclic cell has divided either tangentially or radially, and its daughter cells either radially or tangentially. The cell on its left has divided into two very unequal daughter cells by an oblique wall in the upper corner. Several adjoining pericyclic cells on the right have each divided tangentially.

From a study of serial sections of the primordium shown in fig. 3, it was evident that the first division of the initial pericyclic cell was radial. Each daughter cell then divided tangentially into two unequal cells, the inner one being larger than the outer. The inner left cell must have divided transversely and one of its daughter cells then radially, the other obliquely. Only the cell with the oblique wall shows in the figure. Divisions had proceeded further in the two outer granddaughter cells. The one on the right divided transversely and its upper daughter cell radially into two unequal cells.

In other cases studied the division of the cells of the root origin was radial, or occasionally oblique, but usually the division was tangential. The divisions of the daughter cells may be tangential, transverse, or radial. LEMAIRE (1) and VAN TIEGHEM and DOULIOT (5) found that the initial cells of a primordium always divided tangentially, and that the external layer of daughter cells always divided again tangentially, thereby making three layers of cells which developed into the three regions of the young root. In the work herewith reported no such regularity in development was found.

Cell divisions continue without much enlargement of the daughter cells before their divisions, until the space occupied by the original

one (or several) active pericyclic cells is filled by a large number of small, more or less cubical, meristematic cells (figs. 5–7).

Pericyclic cells adjacent to the initial and parenchymatous cells toward the inside of the initials (fig. 5) begin to divide and become a part of the root primordium. The history of the divisions of these cells is similar to that of the initial cells. In fig. 11, for example, the cell on the extreme left of the primordium has divided either tangentially and its daughter cells radially, or radially and its daughter cells tangentially. The cell lying next to it toward the right has divided tangentially; its daughter cells have divided tangentially. The two inner granddaughter cells divided radially. The cell to the right of the median cell in the primordium shown in fig. 12 has probably divided first radially, then each of its daughter cells tangentially, and each of the granddaughter cells again tangentially, forming two regular radial rows of four cells each.

Further development of the primordium consists in continued cell divisions, enlargement of the cells, and the addition of other neighboring cells (figs. 8–12). It is impossible to trace the order of cell divisions in the older primordia. The cells which resulted from the division of the median initial cell begin first to enlarge (fig. 9). The cells from adjacent initial cells follow, and as enlargement of the cells proceeds the primordium bulges toward the outside (figs. 9–12). The cells of the endodermis divide radially to allow for the push from the developing primordium (figs. 9, 12).

Figs. 7 and 8 show the position of the root primordia in relation to the lateral fibrovascular bundles. The pericycle can be followed outside the primary phloem of the bundle. The interfascicular cambium is continuous with the cambium of the bundle. It is evident that the cambium has taken no part in the formation of the primordium. Several rows of undivided cells lie between the young primordium and the cambium.

The position of the young primordia in relation to the groups of primary phloem cells is best seen in fig. 3.

There is no evidence of differentiation into the epidermis, root cap, cortex, and central cylinder of a root in any of the stages of development previous to that shown in fig. 12. All of the cells of the primordium seem to be equally capable of division. Several nuclei in process of division can be seen in fig. 12.

Fig. 13 shows a root primordium which has become definitely hemispherical and has begun to differentiate into the tissues of a root. The single external layer of cells becomes the epidermis. Two or three layers of cells inside the epidermis become the cortex and the innermost region becomes the central cylinder. A slightly older root (fig. 14) shows the beginning of the root cap from the tangential division of the epidermal cells which surround the apex.

The further development of the root has been adequately described by former investigators. Elongation proceeds by the division of cells in all parts of the young root, and by elongation of the cells of the central cylinder. The endodermis incloses the developing root for a short time, then breaks or dissolves. The cavity opposite the apex of the root (figs. 13, 14) indicates that the cells of the cortex are being dissolved before the growing root. Finally the tissues of the central cylinder differentiate, connection is made with the vascular system of the stem, and the root emerges.

Summary

1. Adventitious roots arising between the fibrovascular bundles from the bases of young cuttings of *Coleus blumei* originate in one to several adjacent cells of the pericycle.

2. The first division of the initial cells of a root is usually tangential, but it may be radial, or even oblique.

3. During the early stages of development the daughter cells divide without first increasing in size, so that a young primordium consisting of many cells may occupy the same space as the pericyclic cells from which it originated.

4. The primordium enlarges and bulges into the cortex before differentiating into the tissues of a root.

5. Subsequent development is precisely as described by the early investigators of the subject of root development.

The writer wishes to express her appreciation to Dr. C. E. ALLEN, Department of Botany, University of Wisconsin, Madison, Wisconsin, for criticisms on the manuscript while in preparation.

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EXPLANATION OF PLATES V, VI

All figures are photomicrographs. The walls of the cells and the nuclei were traced with India ink, and the photographs then bleached. The plates were reduced one-fourth. Figs. 1–12 are transverse sections of stem cuttings; figs. 13 and 14 are longitudinal sections of stem cuttings. All sections of root primordia except fig. 11 are median.

PLATE V

FIG. 1.—Transverse section of stem, showing very young root primordium: *e*, epidermis; *c*, cortex; *en*, endodermis; *p*, pericycle; *pph*, primary phloem; *sph*, secondary phloem; *ca*, cambium; *sx*, secondary xylem; *pi*, pith.

FIG. 2.—Young root primordium.

FIG. 3.—Young root primordium, showing its relation to group of primary phloem cells.

FIG. 4.—Young root primordium; starch grains in endodermis.

FIG. 5.—Young root primordium, showing adjacent pericyclic cells and parenchymatous cells beginning to divide.

FIG. 6.—Root primordium, later stage than fig. 5.

FIG. 7.—Root primordium, showing its relation to lateral fibrovascular bundle.

PLATE VI

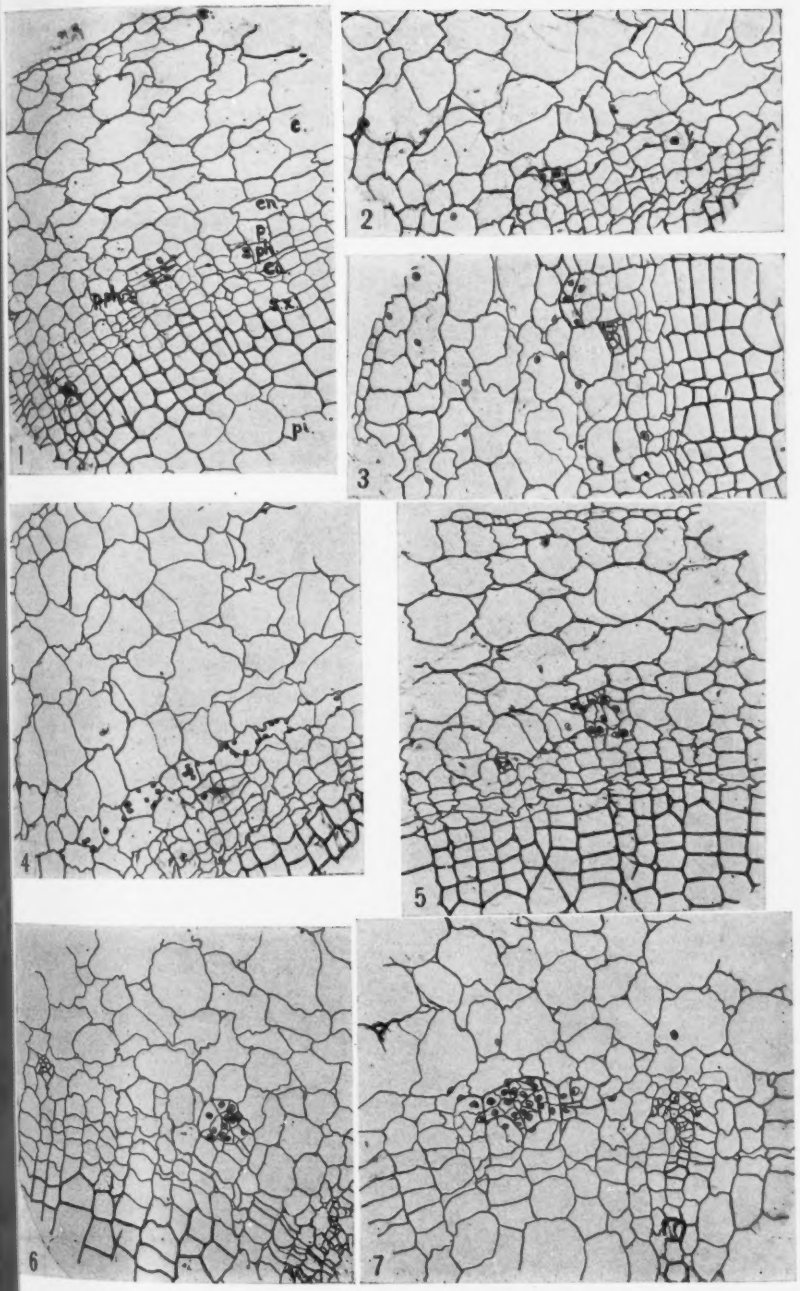
FIG. 8.—Root primordium, consisting of many cells, but occupying little more space than cells from which it originated.

FIG. 9.—Root primordium, beginning to bulge into cortex; endodermal cells bordering primordium have divided; cambium has taken no part in production of primordium.

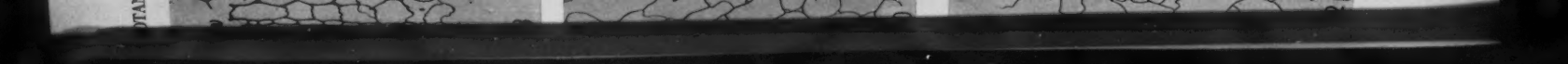
FIGS. 10–12.—Older primordia.

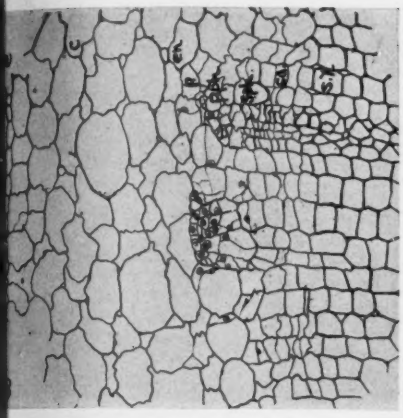
FIG. 13.—Young root in which differentiation into epidermis, cortex, and central cylinder has begun; endodermis and part of cortex dissolved.

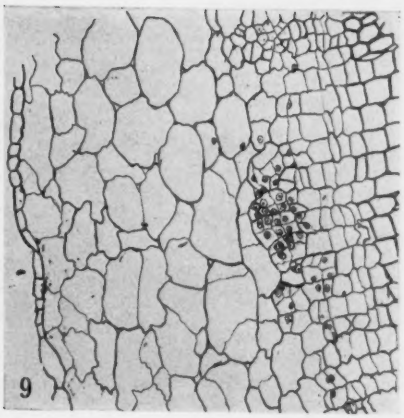
FIG. 14.—Young root, later stage than fig. 13; root cap beginning to form.

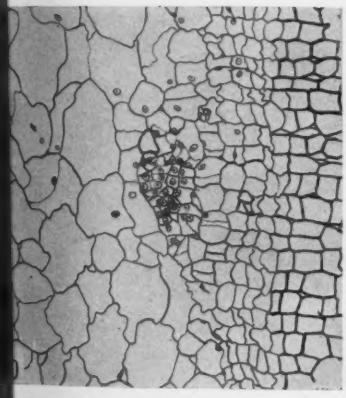


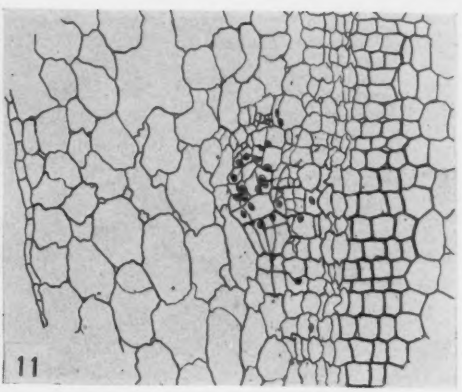
CARLSON on COLEUS

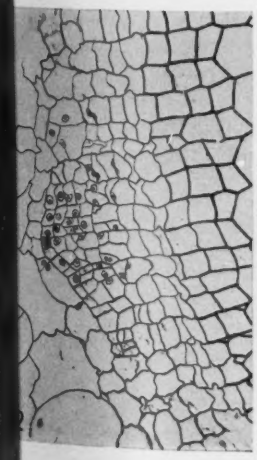


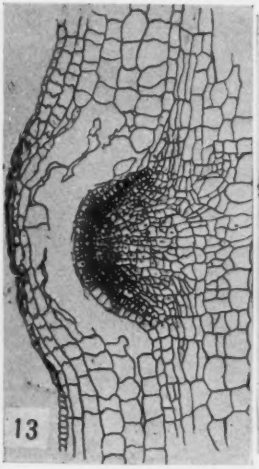


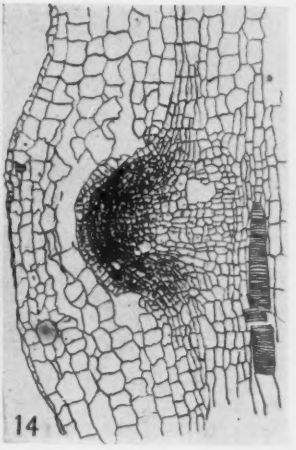












CARLSON on COLEUS

GERMINATION AND VITALITY OF BIRCH SEEDS[1]

HILDA C. JOSEPH

(WITH FIVE FIGURES)

Introduction

In a recent paper, WEISS (13) has given his results with experiments on temperature and medium requirements for the germination of fresh and after-ripened seeds of *Betula populifolia*. He finds that the germination of *B. populifolia* is greatly improved when the seeds are stored in moist granulated peat at low temperature for about two months; that 10° C. is as effective as 5° C. or 0° C. for this purpose; that germination percentage is increased by a treatment with an organic mercury disinfectant previous to stratification; and that such after-ripening at low temperatures results in a marked downward shift in the minimum temperature required for germination.

In the work reported in this paper, the writer has extended studies of the same type to other species of *Betula*, and has also tried to determine whether seeds of the same species vary in their behavior when collected at different times of the season and when kept in different conditions of storage for a year or more.

Material and methods

The main studies have been with *Betula lenta* seeds, which were collected from trees in the forest of the Boyce Thompson Institute for Plant Research. September, October, and November collections were made from the same trees. Since the seeds of the October collection were superior to all others, they were used exclusively in the storage experiments.

The seeds of *Betula populifolia* used in these experiments were also collected from the Institute's forest, while *B. papyrifera* and *B. lutea* were commercial seeds, harvested and shipped by seed deal-

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

ers in October, 1926. All seeds were freed from wings by treatment in a Hobart mixer before being used.

In making the germination tests, Petri dishes with three layers of filter paper were employed, except when another substratum is indicated. Two hundred seeds were placed in each dish.

In the early determinations on the optimum conditions for after-ripening, that is, for the improvement of germination through temporary moist storage at cool temperatures, the seeds were mixed with moist acid granulated peat, neutralized granulated peat, or sand, and placed in large-mouthed bottles covered with cheesecloth.

TABLE I

CHANGE OF CONCENTRATION IN SOLUTIONS OF SULPHURIC ACID USED TO REGULATE MOISTURE CONTENT OF BETULA SEEDS

CONCENTRATION OF H_2SO_4 (PERCENTAGE)	SPECIFIC GRAVITY AT BEGINNING OF EXPERIMENTS	SPECIFIC GRAVITY OF FIRST SET OF SOLUTIONS AT END OF FIRST 2 WEEKS	SPECIFIC GRAVITY OF SECOND SET OF SOLUTIONS AT END OF SECOND 2 WEEKS
Concentrated...	1.8300	1.8046	1.8181
64.8	1.5200	1.5050	1.5024
55.9	1.4300	1.4029	1.4185
43.4	1.3300	1.3042	1.3260
30.4	1.2234	1.2021	1.2092
18.4	1.1210	1.0334	1.1890

In the later studies the seeds were after-ripened in lots of 200 on filter papers in Petri dishes. This latter method proved just as effective and more practical for these studies, since the after-ripened seeds could then be used in germination tests without previous transfer from the bottles to Petri dishes.

The moisture determinations were made by drying non-macerated seeds to constant weight at 103° C. When seeds were prepared for dry storage at various moisture contents, the samples were brought to water vapor equilibrium with a given concentration of sulphuric acid in a desiccator, which was provided with a stirrer for both the air and the sulphuric acid solution. The solutions were changed every two weeks. The changes in specific gravity of the sulphuric acid solutions that occurred during the first two periods are given in table I.

After eight weeks of storage over H_2SO_4 solutions, the seeds had

reached water equilibrium with the solutions. At this time one series of samples was taken out to determine the moisture content of the different lots of seeds; a second series was taken out for the purpose of germination tests; the third and largest amount of material was placed in small bottles, sealed air-tight, and stored under the following conditions: at room temperature, in an ice chest at about 8° C., and in a room varying in temperature from −15° to −8° C. A different method of storage was employed with seeds that had been exposed to humidified atmospheres over sulphuric acid solutions of concentrations lower than 55.9 per cent. Here the seeds were sus-

TABLE II

WEIGHT AND MOISTURE CONTENT OF VARIOUS COLLECTIONS OF BETULA SEEDS AS DETERMINED IMMEDIATELY AFTER HARVEST OR AFTER ARRIVAL OF SHIPMENT

COLLECTION	WEIGHT OF 1000 SEEDS AIR-DRY (GM.)	WEIGHT OF 1000 SEEDS DRIED TO CONSTANT WEIGHT (GM.)	MOISTURE CONTENT IN PERCENTAGE DRY WEIGHT
Betula lenta, collection A and B, September, 1926	0.7300	0.6730	7.54
Betula lenta, collection, October, 1926	0.5471	0.5084	7.09
Betula lenta, collection, November, 1926	0.4863	0.4570	6.04
Betula populifolia, collection, October, 1926	0.0920	0.0853	7.36
Betula papyrifera, commercial collection, 1926	0.2740	0.2502	8.70

pended in a wire basket from the cover of a tightly closed museum jar over the different solutions, and stored in this way at room temperature and in an ice chest. In the low temperature room all seeds were stored in tightly sealed bottles. The total number of seeds of the various species and collections used in these studies approximates one million.

Experimental results

1. EXPERIMENTS WITH NEW SEEDS

It is common practice among gardeners and nurserymen to keep freshly harvested seeds on drying racks for a short time before they are planted. This is done mainly to avoid molding and fermentation in seeds surrounded by a fleshy pulp; but there are also cases in which a period of drying is directly beneficial to the germination

quality of the seed material. Wheat and other grains, especially if they have ripened during a rainy season, are improved by a period of dry storage.

It seemed to be of interest to determine whether such an improvement of germination quality could also be obtained in *Betula* seeds, and whether it could be obtained in seeds picked at the beginning of the harvesting season as well as in those picked at the end. Table II shows that the percentage of hygroscopic moisture of the seeds decreases with the advancing season, and that the weight for 1000 seeds is lower in the later collections.

TABLE III

GERMINATION OF FRESHLY HARVESTED BETULA LENTA SEEDS IMMEDIATELY AFTER HARVEST

DESCRIPTION OF MATERIAL	NO. USED IN EACH TEST	PERCENTAGE GERMINATION AT			
		15° C.	20° C.	25° C.	32° C.
Collection A, September, 1926, from green catkins	500	0	5	3	7
Collection B, September, 1926, from brown catkins	500	0	2.5	5	3.5
Collection, October, 1926	200	0	0	0	17
Collection, November, 1926	200	0	0	1	2

The September collection was taken from closed catkins which were dried in the laboratory to induce shedding of the seeds. The October and November collections were taken from catkins that had already opened on the trees. The November collection had also been exposed to some freezing on the trees. The data in tables III and IV show that the germination quality is improved considerably in all the different collections through one month of dry storage at laboratory temperature. This improvement, therefore, must be due to changes in the seed different from those which are produced by nature through variations in humidity, temperature, and ventilation during the period in which the seed hangs on the tree.

Since acids have been found to have a favorable influence upon the germination of some seeds, it was thought possible that an acid

substratum for freshly harvested seeds might substitute for the beneficial effects of dry storage. Table V shows the negative results

TABLE IV

GERMINATION OF BETULA SEEDS AFTER ONE MONTH OF DRY STORAGE IN LABORATORY

DESCRIPTION OF MATERIAL	NO. USED IN EACH TEST	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alternation of 15°–32° C.
B. lenta, collection A, September, 1926	200	0	0	0	17	48
B. lenta, collection October, 1926	400	0	0	0	41	46
B. lenta, collection November, 1926	200	0	0	0	11	39
B. papyrifera, commercial collection 1926	600	0	9	67	80.5	81
B. populifolia, collection October, 1926	200	0	17	51	60	58
B. lutea, commercial collection, 1926	400	0	2	3	8	15

TABLE V

INDIFFERENCE OF FRESH BETULA LENTA SEEDS TO ACIDITY OF SUBSTRATUM

DESCRIPTION OF MATERIAL	NO. USED IN EACH TEST	SUBSTRATUM	PERCENTAGE GERMINATION AT			
			15° C.	20° C.	25° C.	32° C.
Collection A, September, 1926 from green catkins	500	Filter paper	0	0	0	4.6
	500	Peat pH 4.6	0	5.4	4	6
	500	Peat, neutral with excess $CaCO_3$	0	1	0	5.8
Collection B, September, 1926 from brown catkins	500	Filter paper	0	1	0	5
	500	Peat pH 4.6	0	3	5	3.5
	500	Peat, neutral with excess $CaCO_3$	0	0	4	5
Collection October, 1926	200	Filter paper	0	0	1	17
	200	Peat, neutral with excess $CaCO_3$	0	0	0	2

obtained with tests arranged for this purpose. Except for a probable slight improving of germination on acid peat at 20° C. of the earliest collection of *B. lenta*, there is no noticeable effect that could be interpreted as favorable influence of the organic acids of peat upon the germination quality of fresh seeds.

Similar tests were conducted to determine whether a favorable

effect produced by light, or by increased or reduced oxygen pressure, could be observed, but the seeds were not sensitive to considerable variations in intensity of these factors. A short time later the same experiments were repeated with laboratory-stored seeds with the same negative results.

A decrease of germination percentage occurred in laboratory-stored seeds of *B. lenta* after sterilization with 0.25 per cent uspulun, as shown in table VI.

Although laboratory-stored seeds germinate well at high temperatures, as 32° C. and at alternation of 15–32° C., they do not germi-

TABLE VI

EFFECT OF STERILIZATION WITH USPULUN (0.25 PER CENT FOR ONE-HALF HOUR) UPON GERMINATION OF LABORATORY-STORED SEEDS OF BETULA LENTA; 200 SEEDS IN EACH TEST

DESCRIPTION OF MATERIAL	GERMINATION AT FAVORABLE TEMPERATURE			
	32° C.		Alternation of 15°–32° C.	
Collection October, 1926, sterilized...	68 63 68	Average 66.1	53 50	Average 51.5
Collection October, 1926, not sterilized.....	73 81 74	Average 76	58 59	Average 63.5

nate to a considerable percentage at constant temperatures below 32° C. By a suitable period of moist storage at cool temperatures this behavior can be changed. The seeds are "after-ripened" through this treatment, and afterward germinate better, not only at higher temperatures, but also at temperatures as low as 15° C. If kept in cool storage continuously they finally even germinate at 0° C. All species studied react in a similar way, although *B. papyrifera* is the least dormant of the species studied; and the improvement in total germination percentage, as well as in enlarging of the temperature range favorable to germination, is therefore less pronounced. The seeds of *B. lutea* seemed to be of low vitality from the start, so that differences in behavior under various conditions were less noticeable for this species.

Tables VII–X show the effect that various moist storage condi-

tions have upon the germination of *Betula* seeds at temperatures ranging from 15° to 32° C. Of the four different storage temperatures employed, 0° and 5° C. proved to be very much superior to 10° C., and to a constant frozen condition. This is shown by the high percentage of germination obtained at low germination temperatures

TABLE VII

GERMINATION OF BETULA LENTA SEEDS, COLLECTION OCTOBER, 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

TEMPERATURE DURING PERIOD OF MOIST STORAGE	WEEKS IN MOIST STORAGE	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alternation of 15°–32° C.
Control (no moist storage)		10	0	2	33	67
0° C.	4	80	80	77	72	75
	6	85	72	82	75	81
	8	77	79	81	82	77
	10	82	79	77	76	60
5° C.	4	83	75	77	72	77
	6	70	81	57	72	77
	8	72	77	74	54	56
	10	85	76	87	82	82
10° C.	4	7	22	24	58	78
	6	2	4	25	30	74
	8	10	10	16	54	72
	10	2	4	26	60	71
In frozen condition at −15 to −8°C. (soaked in water for a few hours at room temperature previous to storage)	4	0	0	0	15	37
	6	0	0	1	28	69
	8	0	0	9	22	72
	10	0	2	31	28	55

after a period of moist storage at these temperatures. Seeds stored at 10° C. showed only a slight improvement of germination when transferred to the different germination chambers. For this reason 10° C. cannot be termed a good temperature for after-ripening of the species studied here. This observation differs from that of WEISS (13), who found that for the after-ripening of *B. populifolia* a moist storage period at 10° is as effective as one at 5° or at 0° C. Seeds stored below the freezing point failed completely to after-ripen.

When the samples were put into the cold chamber immediately after they had been placed on moist filter paper, so that no absorption of water could take place before the seeds were frozen, their quality was not altered at all when they were transferred into the germination chambers. When the seeds were allowed to absorb some mois-

TABLE VIII

GERMINATION OF BETULA LENTA SEEDS, COLLECTION NOVEMBER, 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

TEMPERATURE DURING PERIOD OF MOIST STORAGE	WEEKS IN MOIST STORAGE	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alternation of 15°-32° C.
Control (no moist storage)		1	0	4	4	12
0° C.	4	45	22	13	6	17
	6	36	41	28	10	15
	8	Experiment lost				
	10	Experiment lost				
5° C.	4	48	52	42	32	23
	6	42	45	36	29	37
	8	49	50	48	37	35
	10	80	50	48	32	38
10° C.	4	37	22	44	18	45
	6	22	32	41	28	52
	8	46	30	41	28	38
	10	20	34	34	33	45
In frozen condition at −15° to −8° C. (soaked in water for a few hours at room temperature previous to storage)	4	5	8	6	3	38
	6	7	3	6	3	19
	8	5	1	4	4	25
	10	8	8	4	1	55

ture, as in those samples recorded in tables VII–X, some injury could be noted in several samples. The amount of injury increased with the length of time the seeds were soaked at room temperature previous to freezing storage. An alternation of freezing and thawing killed samples of all three species. These results are not in agreement with those obtained by KINZEL (9) for various other seeds. He noted a beneficial effect of freezing and of an alternation between freezing and thawing.

The length of the cool moist storage period does not seem to be

of great importance. Four weeks proved to be long enough to after-ripen seeds at a favorable storage temperature, and ten weeks did not seem to be too long for a favorable result. In all later experiments six weeks of storage at 0° C. was selected as a suitable period for after-ripening.

TABLE IX

GERMINATION OF BETULA PAPYRIFERA SEEDS, COMMERCIAL COLLECTION 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

TEMPERATURE DURING PERIOD OF MOIST STORAGE	WEEKS IN MOIST STORAGE	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alternation of 15°–32° C.
Control (no moist storage)		0	9	12	15.7	16.7
0° C.	4	20	15	21	13	19
	6	24	22	25	19	41
	8	19.5	20	23	15	22
	10	29	20	23	21	19
5° C.	4	38	36	33	26	36
	6	36	35	35	30	34
	8	49	44	41	44	45
	10	53	50	49	39	50
10° C.	4	13	21	26	40	41
	6	19	25	20	33	52
	8	4	8	1	28	45
	10	Not started, material partly infected				
In frozen condition at −15° to −8° C. (soaked in water for a few hours at room temperature previous to storage)	4	0	8	4	7	7
	6	0	1	6	7	7
	8	0	7	4	8	10
	10	0	6	3	7	8

The superiority of the October collection of *B. lenta* seeds over the other collections of the same species, which had been noted in previous tests, became again noticeable when samples of the different collections were after-ripened and then germinated, as is shown in table XI. The conditions under which the October collection was harvested differed from those of the other collections in the following manner: the catkins containing the seeds were dry and partly opened, and a small quantity of the seeds had been shed, while the seeds of the September collection were taken from fresh catkins

which were still closed, and those of the November collection from dried catkins, from which the greater part of the seeds had been shed. The seeds of the November collection had also been exposed to several nights of severe frost, while those of the October collection were harvested before frost had set in. According to this, the optimum

TABLE X

GERMINATION OF BETULA LUTEA SEEDS, COMMERCIAL COLLECTION 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

TEMPERATURE DURING PERIOD OF MOIST STORAGE	WEEKS IN MOIST STORAGE	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alternation of 15°–32° C.
Control (no moist storage)		2	0	5	5	12
0° C.	4	26	27	20	17	13
	6	22	21	17	14	14
	8	20	22	18	11	17
	10	14	19	2	7	0
5° C.	4	5	10	11	12	17
	6	12	7	9	14	12
	8	11	11	10	12	10
	10	10	11	7	9	14
10° C.	4	5	5	7	8	16
	6	4	5	7	8	17
	8	4.5	4.5	6	9	15
	10	4.5	8	9	11	12
In frozen condition at −15° to −8° C. (soaked in water for a few hours at room temperature previous to storage)	4	0	1	5	5	3
	6	2	1	4	6	7
	8	1	1	3	4	7
	10	1	7	1	4	4

time for harvesting *B. lenta* seeds seems to be after the catkins have dried on the tree, but before they have opened far enough for a considerable part of the seeds to be shed.

All these experiments were conducted in the fall of 1926 and the spring of 1927, that is, during the first six months after harvest.

2. EXPERIMENTS WITH STORED SEEDS

In the fall of 1927 a new series of experiments was started for the purpose of determining the keeping quality of seeds from the same collections under various conditions. For these studies seeds

had been stored in open glass bottles at room temperature. They had also been kept, as previously described, with various amounts of hygroscopic moisture at room temperature, and in an ice chest with an average temperature of 8° C.

TABLE XI

GERMINATION OF DIFFERENT COLLECTIONS OF BETULA LENTA SEEDS AFTER-RIPENED FOR SIX WEEKS AT 0° C.; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

COLLECTION	PERCENTAGE GERMINATION AT				
	15° C.	20° C.	25° C.	32° C.	Alternation of 15°–32° C.
Collection A, September, 1926.....	54	62	57	50	67
Collection October, 1926.....	85	72	82	75	81
Collection November, 1926.....	58	44	45	24	30

TABLE XII

GERMINATION OF BETULA SEEDS, STORED DRY AT LABORATORY TEMPERATURE FOR ONE AND ONE-HALF YEARS; SEEDS NOT AFTER-RIPENED; GERMINATION TESTS MADE IN SPRING OF 1928; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

COLLECTION	PERCENTAGE GERMINATION AT				
	15° C.	20° C.	25° C.	32° C.	Alternation of 15°–32° C.
B. lenta, collection A, September, 1926, from green catkins.....	0	0	0	15	50
B. lenta, collection B, September, 1926, from brown catkins.....	0	0	0	7	25
B. lenta, collection October, 1926.....	0	0	4	46	76
B. lenta, collection November, 1926.....	0	0	0	3	16
B. papyrifera, commercial collection 1926..	0	11	15	16	19
B. lutea, commercial collection 1926.....	0	3	1	6	6

The results of the first sets of experiments are given in tables XII and XIII, showing the germination quality of the seeds which had been stored air-dry at room temperature for one and one-half years. They had not been protected against changes in temperature and atmospheric humidity during the entire storage period. Stored seeds, just like newly harvested laboratory-dry seeds, germinated well at 32° C., or an alternation of 15°–32° C., but showed an im-

provement in germination percentage and a great fall in the minimum germination temperature after a period of after-ripening. In figs. 1–3 the percentage of germination of new and stored seeds of various species and collections is compared. It is interesting to note that of the three collections of *B. lenta* made in 1926, the first and second kept their vitality perfectly, while the last collection lost it somewhat. This collection had the poorest quality from the beginning. The reason for this low vitality and poor keeping quality may be due to the period of rainy weather and several nights of frost to

TABLE XIII

GERMINATION OF BETULA SEEDS, STORED DRY AT LABORATORY TEMPERATURE FOR ONE AND ONE-HALF YEARS, THEN AFTER-RIPENED FOR SIX WEEKS AT 0° C.; GERMINATION TESTS MADE IN SPRING OF 1928; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

COLLECTION	PERCENTAGE GERMINATION AT				
	15° C.	20° C.	25° C.	32° C.	Alternation of 15°–32° C.
B. lenta, collection A, September, 1926, from green catkins	52	52	54	41	54
B. lenta, collection B, September, 1926, from brown catkins	20*	18	19	15	17
B. lenta, collection October, 1926	81	83	86	78	77
B. lenta, collection November, 1926	44	34	33	16	25
B. papyrifera, commercial collection 1926	23	19	17	10	15
B. lutea, commercial collection 1926	3	5	4	3	4

* Some fungal infection in this collection.

which these seeds had been exposed on the tree; or to the fact that the heavier and better developed seeds had dropped out of the catkins before this last collection was made.

Of all the species studied, *Betula papyrifera* lost its vitality quickest. In one year of storage the germination dropped from 81 to 19 per cent at the optimum germination temperature. This is interesting in view of the fact that *B. papyrifera* has at the same time the least dormant seeds of the four species. The quality of *B. populifolia* seeds has improved in germination rather than decreased, while that of *B. lutea* is slightly lower.

It has been stated that all freshly harvested seeds improved equally during the first month of dry storage in the laboratory, re-

gardless of the amount of hygroscopic moisture present in the seed at the time of harvest. When the storage period was lengthened,

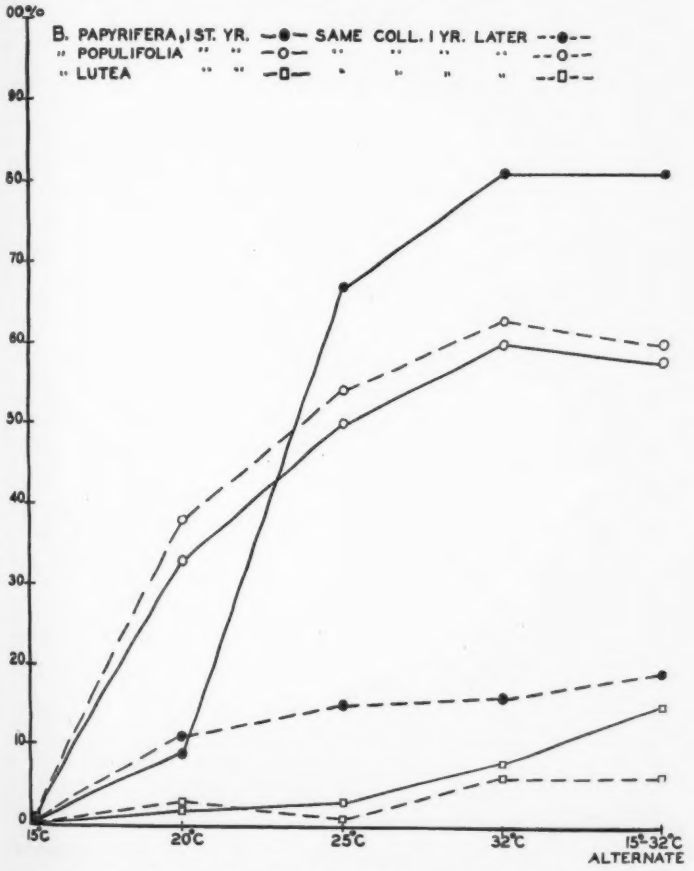


FIG. 1.—Germination of unafter-ripened *Betula* seeds at various temperatures shortly after harvest and after one year of storage at room temperature.

however, the amount of hygroscopic moisture contained in the various samples had a pronounced influence upon the changes going on in the seed during the storage period. Table XIV gives the water content expressed in percentage of dry weight of three species of

Betula as it was obtained by storage over CaO and different solutions of H_2SO_4.

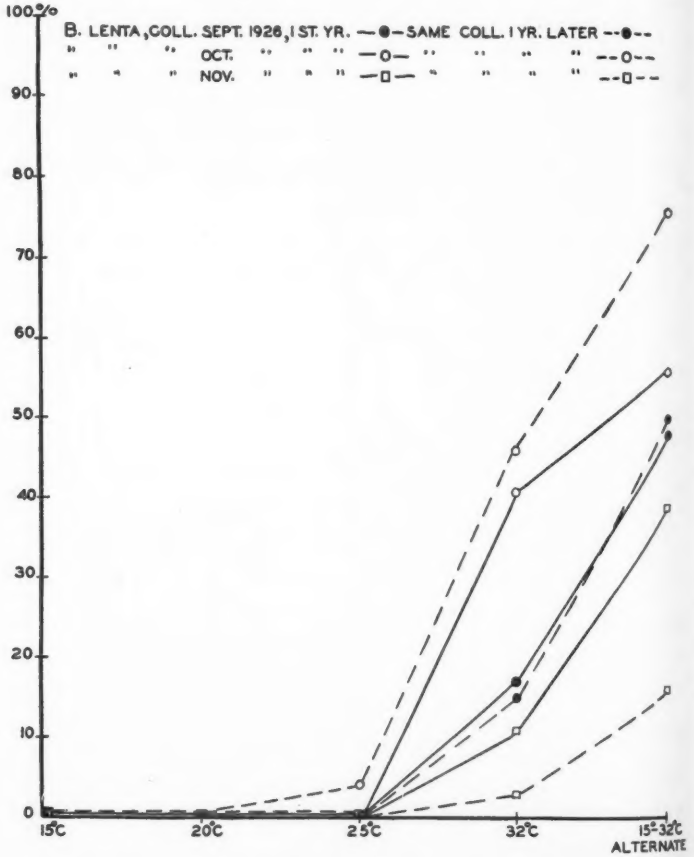


FIG. 2.—Germination of different collections of unafter-ripened *Betula lenta* seeds at various temperatures shortly after harvest and after one year of storage at room temperature.

The keeping quality of each sample from this series was determined for two different storage temperatures, room temperature and ice box temperature, and the results obtained are tabulated in tables

XV, XVI, and XVII. There was also one series of samples stored in a frozen condition at a temperature varying from −15° to −8° C.,

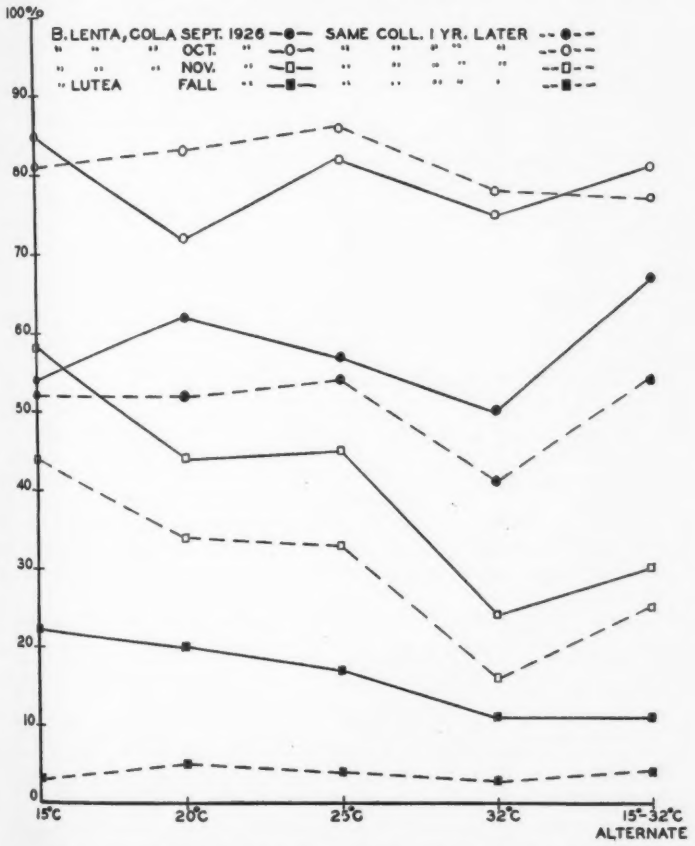


FIG. 3.—Germination of *Betula lutea* and different collections of *B. lenta* seeds at various temperatures shortly after harvest and one year after harvest; all samples after-ripened for six weeks at 0° C. before being transferred to germination chamber.

but since germination tests showed that most of the seeds stored at ice box temperature had not lost in vitality, this last set of samples was left unopened to be tested after a storage period of two years or more. The data in table XV show clearly that seeds of *Betula lenta*,

stored with a moisture content of 8.2 per cent or lower, keep perfectly well during the first year of storage at room temperature. With

TABLE XIV

AMOUNT OF HYGROSCOPIC MOISTURE IN BETULA SEEDS IN EQUILIBRIUM WITH VARIOUS CONCENTRATIONS OF H_2SO_4 AND WITH QUICKLIME

DRYING AGENT	RELATIVE HUMIDITY OVER SOLUTION AT ROOM TEMPERATURE	HYGROSCOPIC MOISTURE IN PERCENTAGE DRY WEIGHT		
		B. lenta, October	B. papyrifera	B. populifolia
CaO	0	0.01	0.6	0.4
Concentrated H_2SO_4	0	0.6	0.7	0.1
64.8% H_2SO_4	10	4.4	4.8	5.2
55.9% H_2SO_4	25	6.3 (original moisture content 7.09)	6.4	7.2 (original moisture content 7.36)
43.4% H_2SO_4	50	8.2	8.7 (original moisture content 8.7)	8.5
30.4% H_2SO_4	75	11.8	11.9	11.5
18.5% H_2SO_4	90	17.6	17.8	17.8

TABLE XV

GERMINATION OF BETULA LENTA SEEDS, COLLECTION OCTOBER, 1926, SEEDS OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE BOX; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

DRYING AGENT	MOISTURE CONTENT	PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE				PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST			
		15° C.	20° C.	32° C.	Alternation of 15°–32° C.	15° C.	20° C.	32° C.	Alternation of 15°–32° C.
CaO	0.01	0	2	62.5	80.5	0	4.5	38	74.5
Concentrated H_2SO_4	0.6	0	4	62.5	73	0	6.5	47.5	74.5
64.8% H_2SO_4	4.4	0	2	53.5	84	0	2	50	79
55.9% H_2SO_4	6.3†	0	0	57	68.5	0	2	69	84
43.4% H_2SO_4	8.2	0	1	32	76	0	0	43	74
30.4% H_2SO_4*	11.8	0	0	0	0	0	0	70	77
18.5% H_2SO_4*	17.6	0	0	0	0	0	0	2	5

* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. lenta*, collected October, 1926, 7.09.

a moisture content of 11.8 per cent seeds do not keep in room temperature, but retain part of their viability at 8° C. With 17.6 per cent

of moisture seeds lose their vitality in low temperature storage as well as at room temperature.

TABLE XVI

GERMINATION OF BETULA POPULIFOLIA SEEDS, COLLECTION OCTOBER, 1926, OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE CHEST; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

DRYING AGENT	MOISTURE CONTENT	PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE				PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST			
		15° C.	20° C.	32° C.	Alternation of 15°–32° C.	15° C.	20° C.	32° C.	Alternation of 15°–32° C.
CaO	0.4	0	26.5	48.5	43	0	17.5	66.5	77.5
Concentrated H_2SO_4	0.1	0	23	46	36	0	8.5	49.5	44
64.8% H_2SO_4	5.2	0	23	73	**68**	0	14.5	57.5	63.5
55.9% H_2SO_4	7.2†	0	38	63	47	0	37.5	82	64.5
43.4% H_2SO_4	8.5	0	12	44	43	0	13	47.5	71.5
30.4% H_2SO_4*	11.5	0	0	0	0	0	22.5	53.5	71.5
18.5% H_2SO_4*	17.8	0	0	0	0	0	2	7.5	9.5

* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. populifolia*, 1926, 7.2.

TABLE XVII

GERMINATION OF BETULA PAPYRIFERA SEEDS OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE CHEST; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

DRYING AGENT	MOISTURE CONTENT	PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE				PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST			
		15° C.	20° C.	32° C.	Alternation of 15°–32° C.	15° C.	20° C.	32° C.	Alternation of 15°–32° C.
CaO	0.6	0	51	64.5	69	1	35.5	70	77.5
Concentrated H_2SO_4	0.7	0	33	63.5	79	0	63	92.5	83.5
64.8% H_2SO_4	4.8	0	12	32	49	0	37	77	77.5
55.9% H_2SO_4	6.4	0	5	8	29	0	78.5	84	91
43.4% H_2SO_4	8.7†	0	3	7.5	11	0	41	81.5	64
30.4% H_2SO_4*	11.9	0	0	0	0	0	26	82.5	80
18.5% H_2SO_4*	17.8	0	0	0	0	0	4.5	6	6.5

* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. papyrifera*, commercial collection 1926, 8.7.

The behavior of *B. populifolia* is somewhat different from that of *B. lenta*, as is shown in table XVI. The keeping quality of *B. populi-*

folia was reduced by storage with a very low hygroscopic moisture, as well as by storage with a water content as high as that of freshly harvested seeds and higher. The optimum moisture content for storage at room temperature seems to be about 5.2 per cent. With seeds kept in the ice box, the importance of the water content decreases for *B. populifolia* in the same way that it does for *B. lenta*.

A third type of reaction is obtained when seeds of *B. papyrifera* are stored with different amounts of hygroscopic moisture (table XVII). Seeds stored at room temperature keep well with greatly reduced moisture content only, lose a considerable amount of vitality when stored with the original moisture content of freshly harvested seeds, and die completely when stored with increased water content.

The three types of keeping quality may be characterized briefly in the following way:

TYPE 1 (*B. lenta*).—At ordinary room temperature seeds keep well with a moisture content ranging from 0.01 to 8.2 per cent (m.c. at time of harvest 7.9 per cent). With a m.c. higher than 8.2 per cent seeds keep only when stored at ice chest temperature.

TYPE 2 (*B. populifolia*).—At ordinary room temperature seeds keep well only at the slightly reduced moisture content of about 5.2 per cent (m.c. at time of harvest 7.2 per cent). With a m.c. reduced below or increased above 5.2 per cent seeds keep well only at ice chest temperature.

TYPE 3 (*B. papyrifera*).—At ordinary room temperature seeds keep well only with a highly reduced moisture content of 0.6 or 0.7 per cent (m.c. at time of harvest 8.7 per cent). With a m.c. above 0.7 per cent seeds keep well only at ice chest temperature.

At a moisture content of about 17.6 per cent all three types lose their vitality completely at room temperature, and almost completely at ice chest temperature, within the first year of storage. A comparison of germination obtained at the optimum germination temperature of 15°–32° C. alternation in all three species of *Betula* stored at various moisture contents in room temperature is given in the curves shown in fig. 4. Fig. 5 shows the modifying influence of a low storage temperature under otherwise similar conditions.

It is of interest to note that although seeds containing a high amount of hygroscopic moisture keep comparatively well at a cool

storage temperature, they do not after-ripen, as is shown by the fact that no germination is obtained at 15° C. as is characteristic for

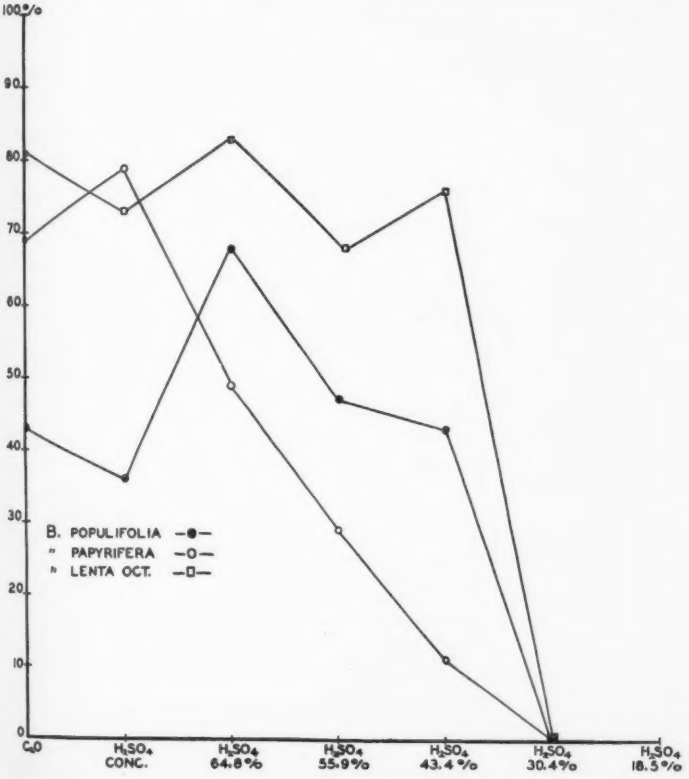


FIG. 4.—Germination of *Betula* seeds after one year of storage at room temperature in hermetically sealed vessels in atmospheres of different humidities; germination at alternating temperatures, 15°–32° C. (eighteen hours a day at 15° and six hours a day at 32° C.).

after-ripened seeds. To secure after-ripening at low temperatures a much higher moisture content of the seeds is required.

Discussion

In a discussion of the results obtained in these experiments, three facts call for special attention: (1) the behavior of freshly harvested

seeds as compared with laboratory-dry seeds; (2) the effect of moist cool storage upon the temperature requirements of germination; and

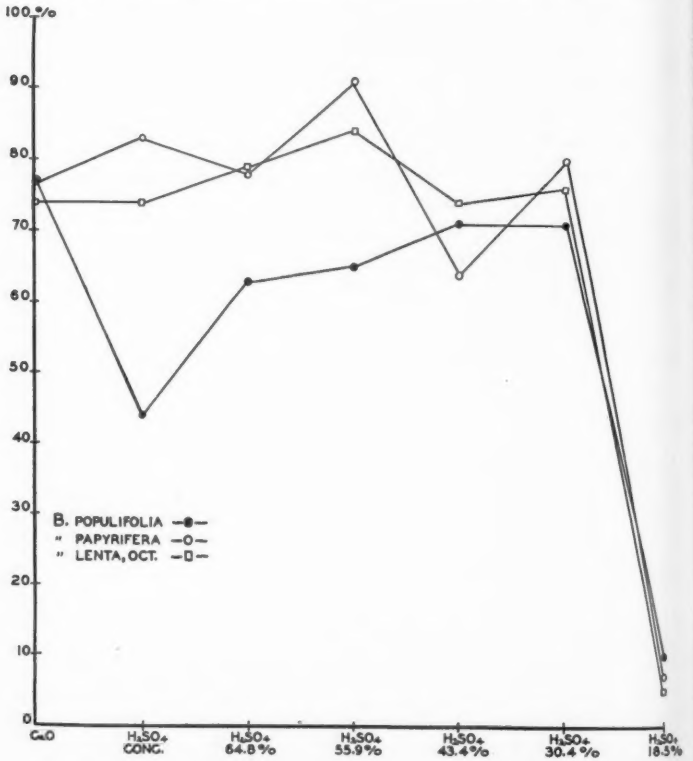


FIG. 5.—Germination of *Betula* seeds after one year of storage in ice chest in hermetically sealed vessels in atmospheres of different humidities; germination at alternating temperatures, 15°–32° C. (eighteen hours a day at 15° and six hours a day at 32° C.).

(3) the relation of moisture content to keeping quality in the different species studied.

It has been shown that fresh seeds of *B. lenta* germinate very poorly immediately after harvest, but improve considerably in germination quality during one month of dry storage. Causes for this improvement may be due to internal changes as well as to an

alteration in seed coat characters. If the changes are internal, they must be different from those that could be produced through variations in moisture content in the seed, or through the influence of frost and other weather conditions while the seeds are still attached to the tree, since different samples of seeds harvested at different times of the season and containing various amounts of hygroscopic moisture were all improved by one month of dry storage. Changes in the seed coat, such as an increase in permeability to water, oxygen, or carbon dioxide produced by a continuous drying at temperatures higher than those prevailing out-of-doors during the harvesting season, may also be responsible for the beneficial effect of dry storage. Unfortunately the small size of birch seeds and the tightness with which the coat fits around the embryo made it impossible to remove the coat of fresh seeds successfully in order to find out whether or not the fresh coat was responsible for the failure of the seeds to germinate.

Many seeds require low temperature stratification to prepare them for germination. This is true of rosaceous seeds (1, 2, 5, 6, and 7), *Tilia* (11), *Juniperus* (10), *Acer* (8), *Cornus*, *Sambucus*, and *Berberis* (3, 4). In all of these high temperatures are ineffective in forcing germination previous to after-ripening in low temperature stratification. *Betula* species differ from this type in so far as they need a period of cool storage only to enable the seed to germinate at the lower temperatures, as 15° and 20° C. At temperatures such as 32° C. and an alternation of 15°–32° C. the seeds germinate without previous treatment. When the samples are kept moist at after-ripening temperatures, such as 0°, 5°, or 10° C. for longer periods than those recorded in this paper, the seeds finally begin to germinate at the storage temperatures, those stored at 10° growing first, and those stored at 0° C. beginning to grow only after 5–6 months.

In this study it was found that the species which required cool storage for later germination at the lower temperatures proved most resistant when stored under various conditions, while the less "dormant" species, *B. papyrifera*, lost its vitality much easier under unfavorable conditions of storage. A physiological or chemical basis for the relation of keeping quality to dormancy has not been worked out.

Conclusion

I. Germination

1. Freshly harvested seeds of *Betula lenta* germinate poorly regardless of time of harvest, moisture content, and dry weight of the seeds at harvest time. The germination quality improves considerably during the first month of dry storage at laboratory temperature.

2. The optimum germination temperature for air-dried seeds of *B. lenta*, *B. papyrifera*, *B. lutea*, and *B. populifolia* is 32° C. constant, or alternation between 15° and 32° C.

3. The minimum germination temperature for dry-stored seeds is remarkably high, being about 30° for *B. lenta*, and about 20° C. for *B. populifolia*, *B. papyrifera*, and *B. lutea*. The dry-stored seeds have a narrow temperature range for germination.

4. When air-dry or freshly harvested seeds of *Betula* are kept in an imbibed condition at low temperatures they after-ripen, or go through a series of changes that improves their germination at high temperatures and enables them to germinate at much lower temperatures. The most favorable temperatures for after-ripening are 0° to 5° C., while 10° is less favorable, and storage in a frozen condition is ineffective or even injurious. Four weeks of stratification at 0° or 5° puts the seeds into condition for excellent germination at 15° C. Six to eight weeks of stratification at these temperatures reduces the minimum germination temperature sufficiently to give good early spring germination in outdoor seed beds. Seeds stratified at 0° C. for 5–6 months begin to germinate profusely even at this low temperature. In short, the minimum germination temperature falls 20°–30° C. with such treatment.

5. Moist sand, granulated peat, or blotting or filter paper are equally effective as stratification media, so that the seeds are indifferent to a considerable range of acidity in the medium.

6. Seeds of *B. papyrifera* are less dormant than are those of the other species studied. They also fall in vitality most rapidly in unfavorable storage condition.

7. The falling of the germination minimum with low temperature stratification must be advantageous to the seeds in nature. The high germination minimum of the freshly shed or dry seeds will insure no germination in the fall. The cold weather of the winter, with the

seeds buried under leaves and snow, will after-ripen them and prepare them for early spring germination. This probably accounts for the abundance of *Betula* seedlings in the early spring. Knowledge of the after-ripening at low temperature stratification is also very important to the producer of birches. Dry-stored seeds sown outside in early spring will not germinate because of the high temperature minimum, while properly stratified seeds will give quick and abundant germination in early spring.

8. The percentage germination of *Betula* seeds reported in this paper is very much higher than that reported in forestry books (12). This is probably easily explained by the facts that past workers have used too low temperatures for the germination of dry-stored seeds, or they have failed properly to after-ripen the seeds that are to be germinated at low temperatures in the seed beds in the early spring.

9. Germination of dry-stored *Betula* seeds is not affected by a considerable range of acidity of the medium in which germination takes place; neither is it influenced by light, increased CO_2, or a considerable range of O_2 pressure. Sterilization with 0.2 per cent uspulun for one-half hour causes a slight reduction in percentage of germination.

II. Storage

10. During one year of air-dry storage at room temperature, seeds of *B. lenta* and *B. populifolia* kept perfectly, while *B. lutea* and *B. papyrifera* fell in viability.

11. The optimum moisture content for seeds stored at room temperature in sealed containers lies considerably below the moisture content of freshly harvested seeds for *B. papyrifera* (0.6 per cent), while *B. populifolia* keeps best with a medium amount of hygroscopic moisture (5.2 per cent). *B. lenta* keeps well in all except very humid conditions during a storage period of one year.

12. Stored at ice box temperature, seeds with higher moisture content keep as well as seeds low in hygroscopic moisture for one and one-half years of storage.

III. Suggestions for growers

13. Birch seeds are harvested to best advantage after the catkins have begun to dry on the trees, but before they have shed a con-

siderable part of the seeds. They should be shaken from the catkins, dried on well ventilated racks, and stored. About six weeks before planting the seeds ought to be stratified in a suitable moist medium at temperatures of from 32° to 41° F. After such a treatment the seeds will germinate in the seed beds in early spring. It takes approximately one month to six weeks for a full stand of young seedlings to appear above the surface of the soil. Early spring planting is recommended.

14. For dry storage of seeds for a few months no special precautions need be taken, but for storage of one year or more the following methods ought to be used: *B. lenta* can be stored in almost any storage room of average room temperature that is dry and well ventilated; *B. populifolia* is sensitive to excessive drying as well as to very high humidities, and should therefore be kept in closed containers at a cool temperature; *B. papyrifera* keeps best when thoroughly dry, which can be done by keeping the seeds suspended in a bag in a closed container, the bottom of which is covered with quick lime.

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AN INEXPENSIVE AND QUICKLY MADE INSTRUMENT FOR TESTING RELATIVE HUMIDITY[1]

WILLIAM B. SHIPPY

(WITH THREE FIGURES)

It is commonly understood that sulphuric acid and various salts may be used in the control of humidity. WILSON[2] discusses the use of sulphuric acid in humidity control, and SPENCER[3] presents a list of inorganic salts supplying a rather wide range of humidities.

Most investigators attempting to control humidity do not actually make humidity readings. The usual procedure is to check the specific gravities of the control solutions from time to time. It is taken for granted that a given concentration of solution will give a specified vapor pressure, but such an assumption may lead to error. To illustrate: A given concentration of sulphuric acid solution under specific temperature and pressure conditions will exert a definite humidity control through desiccation of a limited volume of air. Any modification of these prescribed conditions will necessarily result in a humidity variation of the air under control; hence, any decrease or increase in temperature brings about a corresponding change in vapor pressure. In addition to the preceding, there is often the possibility of leakage in tubing, ill-fitting stoppers, or defective glassware. If living plant material is placed within the controlled vessel, large quantities of water will be given off through transpiration. A further source of difficulty lies with inaccuracies due to the control solution itself, which may contain impurities causing it to give a higher specific gravity reading than would correspond to its sulphuric acid content. Defective recording instruments also might

[1] This work was done in connection with a study sponsored jointly by the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, and the Crop Protection Institute, and published at the expense of the former Institute out of the order determined by the date of receipt of the manuscript.

[2] WILSON, R. E., Humidity control by means of sulfuric acid solutions, with critical compilation of vapor pressure data. Jour. Indus. and Engin. Chem. **13**:326–331. 1921.

[3] SPENCER, H. M., Laboratory methods for maintaining constant humidity. Internatl. Critical Tables **1**:67–68. 1926.

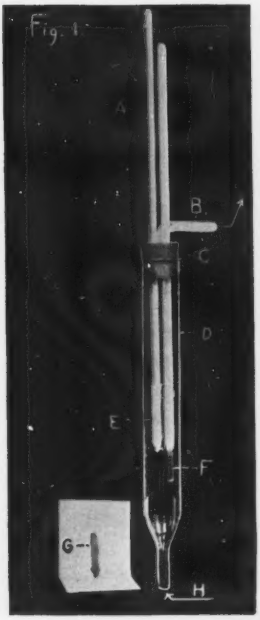
mislead one. In the last analysis it is not the potential desiccation power of the control solution that really matters; it is the humidity actually present within the controlled vessel, and this can only be determined by measurement. Thus it is apparent that difficulties lie in the pathway of any one attempting accurate humidity control. Specific gravity determinations of a control solution are not sufficient evidence under ordinary experimental conditions that a definite vapor pressure has been attained.

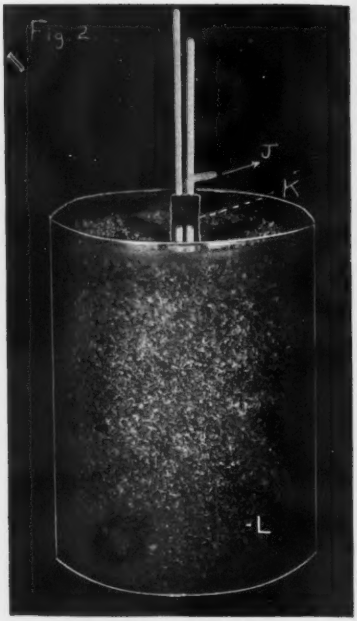
Various instruments are available for humidity testing, including the dew-point apparatus, instruments of the wet and dry bulb principle, and various types of hair hygrometers. These vary in reliability, but among the best are those of the wet and dry bulb type.

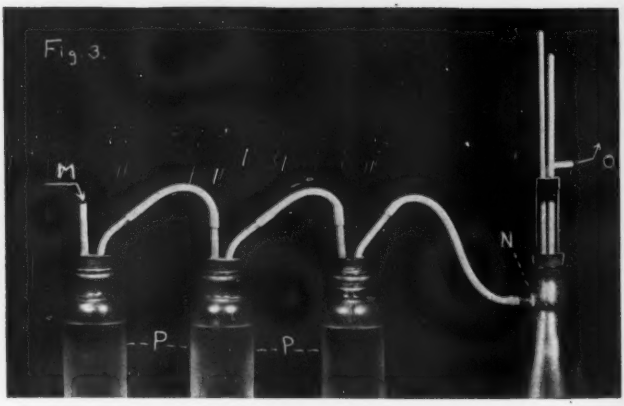
The writer began studies requiring constant humidities during the spring of 1927. It was found desirable to use an arrangement providing for a continuous flow of air through the controlled vessels, and a need arose for some instrument which would check the humidities delivered by the control solutions. The instrument here described, employing the principle of the wet and dry bulb, was designed to fill this need. It is illustrated in figs. 1–3.

An ordinary $8\frac{1}{4}$-inch pyrex test tube (fig. 1D) was drawn out at the bottom and joined with a $1\frac{1}{4}$-inch neck of quarter-inch glass tubing (H). The test tube was fitted with a three-hole rubber stopper (C). Two 100° F. matched thermometers (A) and an elbow of quarter-inch glass tubing (B) were inserted through the stopper. The thermometers were so arranged that when the tube was stoppered, the dry bulb would be approximately three inches and the wet bulb (carefully wrapped with one thickness of clean muslin, which had been thoroughly soaked in water) four inches from the base of the tube. For convenience in holding the instrument, it was commonly placed within an empty suction flask (fig. 3N).

Air forced by compression (applied at M) or drawn by vacuum (applied at O) from the control solutions (P) through this test tube past the bulbs from the bottom inlet (H) to the top outlet (B) gives a reading within a few minutes, the time depending on the rate of air flow. The rate of air flow does not call for great precision, but uniformity of flow is desirable. A flow of one liter per minute (moderately rapid bubbling) is a good rate to use in







FIGS. 1–3.—Fig. 1, instrument for testing humidity: *A*, two matched thermometers; *B*, air outlet tube to which vacuum tube is attached; *C*, 3-hole rubber stopper; *D*, pyrex test tube; *E*, wet bulb; *F*, dry bulb; *G*, fine-mesh copper screen plug; *H*, air inlet tube. Fig. 2, testing relative humidity within solid medium: *J*, point at which vacuum tube is attached; *K*, approximate depth instrument is buried in medium; *L*, peat moss medium. Fig. 3, testing relative humidity of air passing through control solution: *M*, air inlet where compressed air tube may be attached; *N*, empty suction flask used to hold instrument; *O*, air outlet where vacuum tube may be stored; *P*, control solution.

checking most solutions, but satisfactory readings may be made over a fairly wide range. In testing a solution of low vapor pressure, it is not desirable to use too slow an air flow, as the cloth on the wet

TABLE I

TYPICAL SET OF READINGS WITH INSTRUMENT COMPARED WITH THEORETICAL RELATIVE HUMIDITIES AS INDICATED BY SPECIFIC GRAVITIES OF CONTROL SOLUTIONS

OCTOBER 18, 1928; RATE OF AIR FLOW ONE LITER PER MINUTE

DETERMINATIONS BY INSTRUMENT					DETERMINATIONS BY SPECIFIC GRAVITY	
Readings		Depression of wet bulb	Room temperature	Relative humidity[4] (per cent)	Specific gravity of control solution	Corresponding relative humidity[5]
Wet bulb	Dry bulb					
Solution I (water)						
74.5*	75.5*	0.25	77	99	1.000	100
76.75†	77 †					
75.5	77	0.25	77	99	1.000	100
77.25	77.5					
Solution II (sulphuric acid)						
78	80	5.5	79	78	1.190	81
73.5	79					
75	79	4.75	79	81	1.190	81
74	78.75					
77	77.5	4.75	78	81	1.190	81
73.5	78.25					
Solution III (sulphuric acid)						
79	80	8.5	80	68	1.265	65
71.5	80					
75	80	8.5	80	68	1.265	65
71.5	80					
75	79	8.0	78	69	1.265	65
70	78					

* Initial readings.

† Final readings.

[4] MARVIN, C. F., Psychrometric tables for obtaining the vapor pressure, relative humidity, and temperature of the dew-point. U.S. Weather Bureau, no. 235. 78–79. 1915.

[5] WILSON, R. E., Graph on page 328 (article cited).

bulb may dry before time has been allowed for the reading. The reading is made when both mercury columns have come to rest. This makes possible a quick and satisfactory check on the humidity delivered by a control solution. To illustrate how the tester is used, a typical set of determinations on three solutions is shown in table I.

The instrument described can be used for testing relative humidity within solid media. If the investigator desires to know the humidity within a solid medium, as peat moss or a fairly open soil, the basal neck of the tube (H) may be fitted with a plug of fine-mesh copper screen (fig. 1G) to prevent solid particles from entering. The instrument is forced down into the medium (fig. 2L) almost to the top (K), the vacuum tube applied (at J) and a reading made. Such tests are useful in work on the stratification of seeds or in the storage of cuttings, root-grafts, or other similar materials. It may be said in this connection that there is danger of inaccuracy if small volumes of medium are tested, as the replacement of air between the medium particles from the outside would be too great.

One may test with this instrument the relative humidity of large or small volumes of air, 5–10 liters being sufficient for a reading. The size and shape of the instrument make possible its insertion through small openings, such as the hole in the lid of a desiccator or the top of a bell jar. Thus readings can readily be made out of doors or within constant condition chambers of large or small size. In addition to a rather wide use as a tester of humidity, it has the dependability of the sling psychometer. It may be quickly constructed in any laboratory and is made of standard equipment in common use.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

RÔLE OF MOTHER TUBER IN GROWTH OF POTATO PLANT[1]

F. E. DENNY

(WITH FIVE FIGURES)

Introduction

Substances stored in the mother tuber are utilized by the sprout as soon as germination begins, and many experiments have shown that the sturdiness of the sprout, the subsequent rate of growth, and the final yield are related to the size of the seed-piece at the time of planting. Questions relating, however, to the stage of development at which the sprout becomes entirely independent of the mother tuber, the nature of the substances passing to the sprout which are so influential in modifying its growth, the rate at which different compounds pass from the tuber to the sprout, and the correlation in point of time which may exist between this transfer and the subsequent growth of the plant, these questions either find no answer in the literature of the subject or the answers that have been provided are conflicting.

By a series of experiments carried out in 1926, 1927, and 1928, it is believed that additional information upon these points has been obtained. The method of study has been, in brief, to amputate the mother tuber (by a method which involved a minimum disturbance of the root system) at different intervals after planting, that is, at different stages in the development of the plant, to observe the effects of this amputation upon the subsequent growth and final yield by comparison with check plants from which mother tubers had not been removed, to determine by chemical analyses of the amputated mother tissue what substances had disappeared and at what rate, and finally to make use of these observations to determine the periods at which connection with the mother tuber is critical,

[1] Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

and to correlate the chemical composition at any period with behavior after amputation.

In these experiments an effort was made to deal with sufficiently large numbers of plants so that the influence of experimental errors would be reduced. During the three years, about 3000 plants, including amputated and checks, have been used in the tests. Consequently the samples of tissue taken for analyses have represented large numbers of individual tubers, and, since the yield data were obtained separately for each plant, the influence of variability (which is high in experiments on potato yield) could be measured and allowed for. Furthermore, although only two varieties, Irish Cobbler and Bliss Triumph, were used in the main experiments, preliminary tests were also made with ten other varieties, and it is likely that the results can be considered applicable to a considerable number of varieties. In one respect these experiments have been narrowly restricted, however, in that in nearly all cases only one size of seed-piece has been used, namely, 28 gm. The influence of size of seed-pieces upon these results requires further experimentation.

Summary of literature

The attempt is not made here to deal chronologically with the various papers that have been published on this and related subjects, but rather to indicate the relation which they bear to the particular phases concerned in the present experiments, and to show the status of the subject at the time the work was initiated.

Amputation experiments were carried out by SIKORSKI (18), who on May 20 planted six potato tubers (weight 63–66 gm.), three in soil and three in sand. He removed the mother tuber from one plant in each series on June 30, and from another plant on August 31. As compared with plants from which mother tubers were not removed, it was found that amputation had reduced the yield in all cases. He believed that the influence of the mother tuber is not restricted to the early stage of development, but extends over the complete vegetative period. He suggested that in the later stages the mother tuber acts as a storage organ for water, and that this function is very important in periods of drought.

More recently SELIBER (16, 17) has furnished two reports of his

amputation experiments, in which larger numbers of plants were dealt with. His method of removing the mother tuber from the growing plant was to undermine the plant by removing earth until the mother tuber could be removed by hand. Two types of check plants were used, one being plants that were undermined in the same way until the mother tuber was touched by the hand but was not removed, and the other being plants which grew continuously in the soil without disturbance. He found that the removal of the mother tuber, even in midseason, reduced the final yield; but that the variety of potato was an important factor, the varieties Reitan and Chugunka being influenced to a great extent by amputating the mother tuber, while in the varieties Kruger and Epicurez the substance of the mother tuber was not utilized. With these latter varieties, presence or removal of the mother tuber was not a matter of importance, so far as the yield was concerned. SELIBER also discusses the importance of the mother tuber as a water storage organ.

The literature relating to the extent to which the substance of the mother tuber is utilized, that is, as to the amount of reduction in dry weight during the growing season, is conflicting. The earlier workers were impressed by the large proportion of dry substance that is removed from the mother tuber, while certain more recent reports tend to show that considerable amounts of the stored foods remain in the seed-piece even at the end of the growth period.

As an example of the first group, we may cite FITTBOGEN, GROENLAND, and FRAUDE (9), who carried out the first and most complete set of analyses of mother tubers at intervals during the growth of the plant. They planted the tubers on April 13 and dug up sample lots of plants on May 28, June 18, July 2, July 25, August 20, and September 22. The analyses of the mother tubers on these dates showed a rapid decrease in protein, starch, fat, "undetermined organic," and ash. More than 70 per cent of the organic substance of the mother tuber had been lost by June 18.[2]

MÜLLER (12) noted that even at the end of a dry summer the mother tubers that were recovered from potato vines at harvest were not shrunken, but were full of sap. But his analyses of the recovered

[2] Their analyses show that in spite of this loss of dry matter, the sugar content of the mother tubers increased, and the present experiments confirm this finding.

seed-pieces showed that the dry substance represented about 3.7 per cent of the fresh weight, that almost all of the starch, protein, and phosphorus compounds had disappeared, and that ash constituents (except KCl!) had been reduced to about one-half the original amount.[3]

In one of the more recent articles relating to the utilization of the stored food, JONES and ROSA (10) state:

> The analyses of RAMSEY and ROBINSON (1917) show that carbohydrate, N, and ash constituents of the mother set are far from exhausted, even at the end of the growing season.

Still more recently we have the report of LUDWIG (11), which contains data on the composition of the mother tuber after growth in culture solutions. His experiments showed that after the sprouts had become 6, 8, or even 30 cm. high, with normal dark green leaves, and with roots and stolons 20 cm. long, the mother tubers still retained 40 per cent of their total nitrogen and about 65 per cent of their carbohydrate reserves. He emphasizes the "common observation" that eyes dug out of tubers will produce normal plants with tubers not inferior to those produced by seed tubers planted in the usual way. He states that, during the late war, farmers used not only pieces of tubers, but also merely well developed eyes attached to pieces of potato peelings, and these gave normal yields. The failures that had resulted from the use of cut tubers were thought to be due, not to the small quantity of reserve food in the seed-pieces, but to loss by rotting.

DE VRIES (6) studied the transfer of food reserves from the mother tuber to the sprout at different stages of growth. These observations were largely qualitative, no analyses being furnished and no amputation experiments being carried out. His opinion was that the mother tuber furnished nutrients to the sprout until germination was completed (plants above ground with green leaves), but that after the beginning of formation of young stolons the food supply was thenceforth transferred only to the newly forming tubers.

[3] One paragraph in MÜLLER'S article is significant in connection with the present experiments. He states: "It would be interesting to carry out a special experiment to determine in what manner the absolute weight of the mother tuber changes during the vegetative period. Also, how the yield of young tubers would be influenced by removing the mother tuber after the sprout had completely developed."

Although, as will be shown later, the evidence is in favor of the older view that the depletion of the food reserves stored in the mother tuber is extensive or nearly complete, there is no agreement as to the nature of the substances furnished by the mother tuber that are so influential in increasing growth. APPLEMAN (1), by a series of experiments on the relation between the size of the seed-pieces and the strength of the sprout, showed that when the size of the seed-piece fell below a certain minimum, the vigor of the sprout decreased as the size of the seed-piece was reduced. He believed that the failure of small pieces to produce good sprouts was not due primarily to the lack of usual food materials, "as sprouts on pieces still large enough to contain an abundance of these substances show considerable decrease in vigor." He concluded "that the potato tuber contains a limited amount of a special growth promoting substance, and if the amount of tissue surrounding the growing bud is too small there is not enough of this substance available for normal growth." The view expressed by JONES and ROSA (10) is that "the large set furnishes something to the plant arising from it, which is not present in sufficient amount in the smaller set."

From this short summary of the literature, it is apparent that further information is needed upon the subject. In previous amputation experiments either not enough plants have been used, or not enough stages of development of the plants have been included in the test to give a complete account of the relation of the mother tuber to the sprout. The observations on the effect of the removal of mother tubers have not been accompanied by chemical analyses of the amputated tissue. The data as to the extent of the utilization of the mother tuber show sharp disagreements, and further analyses are needed to show which of these divergent views is correct. The present experiments have provided additional information upon several of the questions involved, but a discussion as to the bearing of the results upon various phases of the problem will be postponed until the experimental results themselves have been described.

Results

The experimental results may be divided into two parts, the first dealing with the amputation of the mother tubers at intervals after planting and its effect upon subsequent growth of the plant, the

second dealing with the chemical composition of the mother tissue that was removed at different periods of growth.

Amputation of mother tuber and its effect

Method of removal from sprout.—Since it was essential that the mother tuber be removed from the sprout with as little disturb-

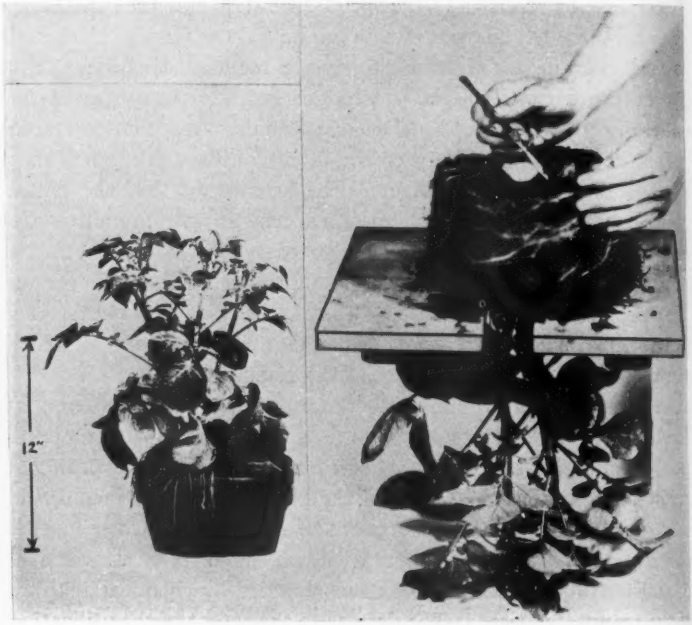


Fig. 1.—Method of amputating mother tuber from sprout: left, plant grown in pot buried in soil; right, plant inverted, placed upon board with slot from edge to center used for support; pot then discarded. Note mother tissue being removed piece by piece with scalpel; plant placed in soil after removal of mother tuber.

ance of the root system as possible, the plants were grown in clay pots which were planted in the soil, the top of the pot being slightly below the surface of the soil. At any subsequent period of growth at which it was desired that the mother tuber be removed, a number of pots containing the now well-rooted plants were removed from the soil and inverted (fig. 1). The mother tuber, which had been

planted in the bottom of the pot just above the drainage hole, was now freely exposed and could easily be detached by cutting; the pot was then discarded and the plant was replaced in the soil, and from this time onward grew under normal conditions, that is, undisturbed by the restrictions of growth in pots.

At each stage of development at which mother tubers were removed from a certain number of plants, an equal number of plants were removed from pots and planted in the soil, but from these the mother tubers were not removed. These were considered as check plants, since they were handled in precisely the same way except with reference to the removal of mother tubers. It is true that there would be somewhat greater disturbance of the soil in removing the mother tubers than would result in handling the checks, but an attempt was made to equalize this difference partially, by purposely disturbing the soil around the mother tubers in the check to about the same degree.

In setting the plants in the soil, the arrangement of the amputated plants and their corresponding checks depended upon the stage of development at which the mother tubers were removed. When they were amputated at early stages, for example, at stages 1 and 2 (fig. 2), the plants were arranged in the field in sets containing five plants each, that is, five plants of the amputated series, then five plants of the check series, then five plants of the amputated series, etc. At the later stages of growth, for example, at stages 3 and 4 (fig. 2), however, the amputated plant and the corresponding check were planted side by side, in order to equalize soil differences as nearly as possible and to permit more dependable comparisons.

It was found that the plan of having a set of checks for each set of plants from which mother tubers were removed, and repeating this at each stage of growth at which they were removed, was essential in order to permit dependable comparisons at the end of the season. In the first year of the experiments (1926) this precaution was not taken, the "checks" being merely plants which had never been in pots at any part of the season and from which mother tubers were not removed. The yield data at the end of the season did not therefore take into account the influence of the growth in pots, as compared with growth in free soil, up to the time of removal of

mother tubers. Consequently the yield data for 1926 had to be rejected. In 1927 and 1928, however, the amputated lots and the corresponding check lots grew for the same periods in the same sized pots, and for the same periods in the soil, the only variable factor being presence and absence of the mother tuber.

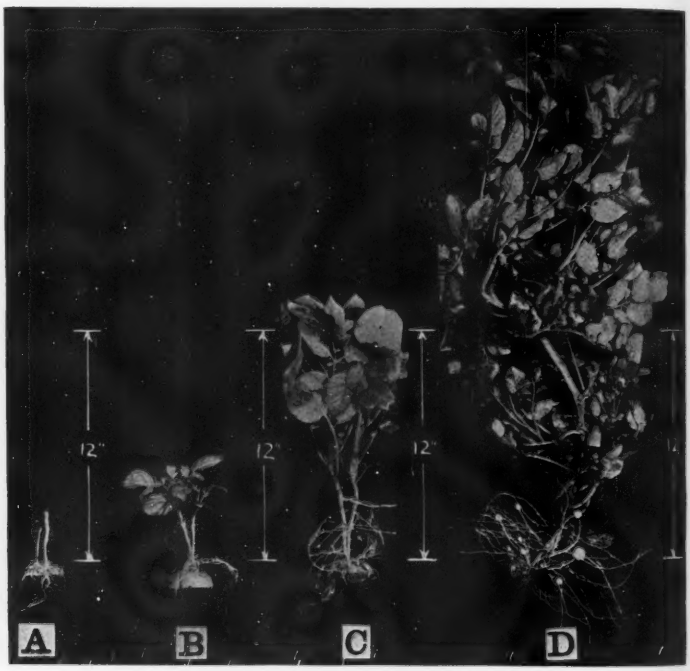


FIG. 2.—Conditions of plants at four different stages at which mother tubers were amputated: *A*, stage 1; *B*, stage 2; *C*, stage 3; *D*, stage 4.

The preliminary experiments in 1926 also showed that the exact method of removing the mother tuber from the plant was a matter of considerable importance. When it was removed by cutting across the stem at the surface of the tuber, or by forcibly twisting it off from the stem, bleeding at the cut end of the stem occurred. This often resulted in wilting of the plant, even when the well-rooted plant with undisturbed root system was placed at once in soil to

which an abundant amount of water had been added. This difficulty was obviated in 1927 and 1928 by sectioning off small portions of tissue from the mother tuber piece by piece, until finally a small piece of it was allowed to remain at the base of the stem. Under these conditions no wilting was observed. The method of removing the mother tuber is shown in fig. 1, and the condition at the base of the stem after amputation was completed is shown in fig. 4 (see left-hand plant at K).

SIZE OF SEED-PIECE AND VARIETIES.—Seed-pieces weighing 28 gm. each were used, and in order to be assured of uniformity in this respect the pieces were weighed individually, and trimmed until the weight did not vary by more than 1 gm. In the 1926 and 1927 experiments only Irish Cobbler variety was used, but in the 1928 work Bliss Triumph was included in the main experiment. In this year also, a preliminary test was made with a number of other varieties, as shown in table VI, in order to note the behavior of various varieties with respect to utilization of the dry matter in the mother tuber.

SIZE OF POTS.—For the series in which the mother tubers were to be removed at stage 1 (fig. 2), clay pots 3.5 inches in diameter and 3.5 inches deep were used; for the series at stage 2 the pots were 6 inches by 4.75 inches; for the series at stage 3 they were 8 inches by 4.5 inches; and for stage 4 two sizes were used, 10 inches by 4.5 inches and 12 inches by 4.5 inches.

STAGES OF DEVELOPMENT AT WHICH MOTHER TUBERS WERE REMOVED.—The mother tubers were removed at four different stages of development, and the sizes of the plants at each amputation stage are shown in fig. 2. At stage 1 (A) the mother tubers were removed when the sprouts had just pushed through the soil. The tips were green, but the young leaves had not yet expanded. This was the condition 22 days after planting. At stage 2 (B) the mother tubers were removed when the sprouts were about 2 inches above the surface of the soil and the leaves fully expanded. This was the condition of the plants 29 days after planting. At stage 3 (C) the plants were about 10 inches above the surface of the soil, were making a vigorous growth, underground stolons several inches long had formed, and young tubers were well started in development. These plants might be termed half-grown. This was the condition 42 days after planting.

FIG. 3.—History of development of amputated and check plants during growth period when mother tuber was removed at stage 1: *E*, amputated (left) and check (right) at time of amputation, May 24; *F*, two plants of same series showing condition of amputated and check plants on June 8; *G*, further stage in growth of amputated (left) and check (right) plants as shown by representative plants on June 19; *H*, comparison of amputated (left) and check (right) plants on July 31.

At stage 4 (D) the plants were about 24 inches above the surface of the soil, had attained approximately the maximum height of vine, were in full bloom, and many young tubers were fully formed. This was the condition 56 days after planting.

SUBSEQUENT GROWTH OF PLANTS AFTER AMPUTATION.—The growth of the plants from which the mother tubers were removed as compared with the growth of the corresponding check plants is shown in figs. 3 and 4. These photographs were obtained by selecting what appeared to be average plants from each group, and placing the treated and check plant side by side for photographing. Because of the great variability of potato plants it will be recognized that only rough comparisons can be obtained in this way, since a single plant cannot be depended upon to show accurately the average condition of a group.

The subsequent growth of the plants from which mother tubers were removed at stage 1 is shown in fig. 3. The condition of the amputated plant and its check on the day of the removal of the mother tuber (May 24) is shown at E; and F, G, and H show the conditions of the plants on June 8, June 19, and July 31 respectively. It will be observed that the plants from which the mother tubers were removed made slow growth at first, but that later in the season a surprisingly large amount of vine was developed. The amputated plant shown in fig. 3 (H, left) shows a comparatively larger quantity of tubers, however, than is justified by the final yield data shown in table IB, thus indicating that although the plant used for the photograph showed an average condition of vine, the underground condition was not truly representative of the group. At any rate it is apparent that removing the mother tubers at this early stage influenced both the subsequent vine growth and tuber yield, and that the effect was greater upon the yield of tubers than upon the final size of vine.

The subsequent growth of the plants from which mother tubers were removed at stage 2 is shown in fig. 4. The condition of the amputated plant and its check (May 31) is shown at K, and the condition on June 19 is shown at L. From this date onward the amputated plants grew rapidly enough to overtake the checks, so that later in the season no difference in size of vine could be observed between amputated and check plants, and consequently no further photo-

graph in this series was made. As for the plants at stages 3 and 4, the condition of the checks is shown in fig. 2, C and D. The subsequent rates of growth of the vines by amputated and check plants in these two series were so nearly equal that no difference was observable and further photographs were not made. But, although the amputation at these stages did not produce an observable effect upon

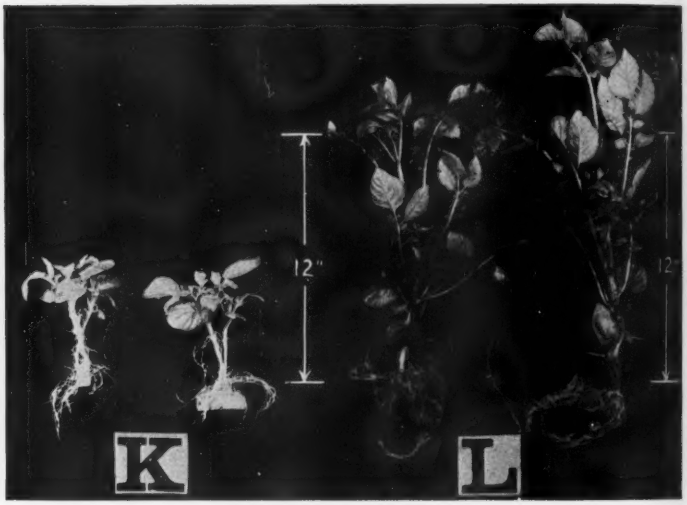


FIG. 4.—*K*, condition of amputated (left) and check (right) plants at time of amputation (May 31) when mother tubers were removed at stage 2; *L*, representative samples of same series on June 19; later in season the vine growth of amputated and check plants approximately equal.

the growth of vines, it will be seen from the data in table IB that it is possible that the yield of tubers was influenced, either unfavorably as in the case of Irish Cobbler, or favorably as in the case of Bliss Triumph.

YIELDS OBTAINED.—Although a yield record was obtained in the 1926 experiments, these data are not included in this report because of the failure to obtain adequate checks, as described in a previous paragraph. The plants in the 1927 experiments suffered from attacks of aphis, and the growth of many of the plants from midseason until the vines died was not normal, many plants dying and other plants

being obviously injured by the numerous spray applications that were needed. This resulted in many missing hills in the field; and, since in these experiments the arrangement of the plants in the field was a necessary feature in permitting dependable comparisons as to the final yield of amputated and check plants, the yield data for 1927 were excluded. In the 1928 experiment (which is to be considered the main one in this series), however, the conditions were favorable in all respects for normal growth; and, since the amputated lots had comparable checks in each case, the data are considered reliable and are presented in tables IA and IB.

In table IA the first four columns show the yield in grams obtained from plants from which the mother tubers were removed at stage 1, that is, when the sprouts were first emerging from the soil. Columns 1 and 2 show the results with Irish Cobbler, and columns 3 and 4 show those with Bliss Triumph. Each figure represents the yield in grams from a single plant. The average yields when mother tubers were not removed were 255 gm. for Irish Cobbler and 350 gm. for Bliss Triumph, while the yields per plant when mother tubers were removed were 86 gm. for Irish Cobbler and 113 gm. for Bliss Triumph. Table IA, columns 1, 2, 3, and 4 show that removing the mother tubers at the time of emergence of the sprout markedly reduced the yield. Furthermore, it will be noted (columns 2 and 4) that many of the plants from which mother tubers were removed at this early stage failed to develop at all. At this period of development the presence of the mother tuber is critical.

The yield data for stage 2 are shown in table IA, columns 5, 6, 7, and 8. Here again each figure represents the yield from a single plant. Columns 5 and 6 show that the average yield for Irish Cobbler was 328 ± 12.2 when the mother tuber was left on, and 274 ± 9.3 when it was removed. The difference (54 gm. per plant) is 3.5 times its probable error, and is therefore significant, showing that the removal of the mother tuber at this stage reduced the yield. In the case of the Bliss Triumph (columns 7 and 8) the corresponding values are 244 ± 11.4 for plants with mother tubers left on, and 197 ± 11.8 for plants with them removed. The difference (47 gm. per plant) is only 2.86 times its probable error, and is insufficient to show that amputation reduced the yield. Further experiments with this varie-

ty at this stage of development are necessary. It is apparent that the planting plan must be such as to place the amputated plants and their corresponding checks side by side in the field, in order to permit

TABLE IA

EFFECT OF AMPUTATION OF MOTHER TUBERS UPON YIELD

MOTHER TUBERS REMOVED AT TIME OF EMERGENCE OF SPROUT (STAGE 1, FIG. 2A)				MOTHER TUBERS REMOVED AFTER GERMINATION WAS COMPLETE AND LEAVES UNFOLDED (STAGE 2, FIG. 2B)			
IRISH COBBLER		BLISS TRIUMPH		IRISH COBBLER		BLISS TRIUMPH	
Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.
115	40	493	59	449	358	370	258
255	46	198	118	527	202	357	91
350	40	102	140	385	282	385	190
343	75	418	42	447	290	260	109
470	35	474	74	*	*	*	120
292	27	403	80	257	390	115	330
222	25	275	*	468	270	190	155
215	26	177	*	268	276	207	243
290	*	354	*	170	290	170	257
143	*	253	*	240	248	107	225
430	105	307	*	277	294	270	44
235	208	465	*	294	295	252	29
230	*	454	*	257	360	329	62
85	*	193	*	380	199	208	85
129	*	330	*	393	320	240	190
288	120	318	230	306	440	440	185
287	75	393	42	520	440	207	285
355	97	615	72	340	330	170	285
273	*	192	185	370	415	472	300
75	160	329	146	520	249	180	*
203	44	474	172	340	362	390	192
318	145	490	126	209	290	235	375
270	150	*	98	228	192	250	176
*	128			195	290	172	115
		Average 350	Average 113	256	190	290	390
Average 255	Average 86			302	100	70	250
				308	222	190	202
				153	279	185	70
				162	142	308	*
				274	147	*	*
				340	247	135	77
				330	173	290	310
				347	244	123	270
				456	241	215	135
				390	257	254	304
				Average 328 P.E. ±12.2	Average 274 P.E. ±9.3	Average 244 P.E. ±11.4	Average 197 P.E. ±11.8

* Plants failed to develop; missing hills not included in computing averages and probable errors.

more critical comparisons regarding the influence of amputation at this stage upon yield.

The yields for the series in which the mother tubers were removed at stage 3 (when plants were half-grown) and stage 4 (when plants had attained maximum height and were in bloom) are shown in

table IB. Since the two varieties behaved quite differently with respect to the effect of the removal of mother tubers at these two stages, they will be discussed separately. The results with Irish Cob-

TABLE IB

EFFECT OF AMPUTATION OF MOTHER TUBERS UPON YIELD

(CONTINUATION OF TABLE IA)

MOTHER TUBERS REMOVED WHEN PLANTS WERE 10 INCHES HIGH, AND YOUNG TUBERS FORMING (STAGE 3, FIG. 2C)				MOTHER TUBERS REMOVED WHEN PLANTS WERE NEARLY MAXIMUM HEIGHT, AND IN BLOOM (STAGE 4, FIG. 2D)			
IRISH COBBLER		BLISS TRIUMPH		IRISH COBBLER		BLISS TRIUMPH*	
Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.
200	105	135	253	130	154	85	200
160	154	140	170	117	30	58	68
192	290	122	125	106	47	106	124
59	130	242	200	183	146	182	125
58	200	202	208	135	180	125	155
140	177	150	249	134	225	142	95
402	226	302	275	102	187	232	198
193	160	186	253	247	107	138	116
222	290	333	264	251	190	140	167
270	265	274	262	118	155	166	196
240	170	116	180	173	204	90	180
250	70	106	366	155	170	148	190
140	254	150	144	135	130	210	234
253	215	176	294	196	142	116	154
150	196	78	157	103	103	160	225
223	105	127	201	112	117		
190	144	252	93	103	52	Average 140	Average 162
206	172	156	115	56	111		
220	198	135	89	136	216		
308	70	60	106	200	172		
268	142	256	240	204	118		
290	120	146	153	183	216		
126	203	152	180	189	207		
235	190	40	250	177	255		
224	150	198	150	311	280		
190	135	103	120	177	230		
100	102	69	53	126	208		
120	70	142	233	235	197		
174	90	173	56	178	210		
120	90	131	121	218	154		
190	108	117	99	157	172		
192	122	133	107	202	218		
148	130	181	212	158	147		
125	146	90	156	230	223		
190	220	125	159	187	253		
153	190	128	206	253	186		
73	202	44	176	162	202		
240	127	140	220	138	155		
130	207	161	163	110	165		
185	75			133	112		
170	176	Average 153	Average 181	88	206		
204	206			228	212		
243	148			105	135		
155	190			179	62		
170	277			45	78		
				102	109		
Average 189	Average 166			Average 160	Average 164		

* Because of rotting of mother tubers of Bliss Triumph between stages 3 and 4, only 30 plants with firm mother tubers were available for amputation at this stage.

bler for stage 3 are shown in columns 1 and 2, table IB, and show the response from 45 pairs of hills, one plant of each pair being the check from which the mother tuber was not removed, and the other being the plant from which the mother tuber was removed at stage 3 (plants half-grown, fig. 2C). The average yield per plant when mother tubers were left on was 189 gm., and that for plants from which mother tubers were removed was 166 gm.

It was anticipated that probably only small differences would be found in the yields from plants with mother tubers not removed, as compared with plants from which they were amputated at these later stages of growth. Consequently, in transplanting after removal of mother tubers in the two series at stages 3 and 4, the amputated and check plants were placed adjacent to each other in pairs, in order that the soil conditions would be more nearly alike, and in order that the yield data could be compared by the simultaneous difference method employed by ENGLEDOW and YULE (**7**) and by use of the formula given by FISHER (**8**). Calculations made by ENGLEDOW and YULE's method show odds of only 13 to 1 for significance of the observed difference in this case. When the FISHER formula is applied to the same data, the value of "t" found was 1.77, which, as shown by the table for "t" values (FISHER **8**, p. 137), corresponds to odds of less than 20 to 1. FISHER'S requirement for a probability of $P = 0.05$ would necessitate a value of about 2.0 in this case; the value actually found (1.77), therefore, falling short.

Taking into account the probabilities shown by these two methods, it seems that, while there is some evidence that the removal of mother tubers decreased the yield, the assurance is not complete, and further experiments must be made to permit a definite statement as to the influence of removal at this stage of development.

The response of Irish Cobblers to the amputation of mother tubers at stage 4 (plants nearly full height and in bloom) is shown in table IB, columns 5 and 6. In this case the average yield with mother tubers left on was 160 gm. per plant, and with them removed was 164 gm. Removing the mother tubers at this late stage of development had no effect upon the final yield of tubers.

The results obtained with Bliss Triumph variety at stages 3 and 4 are shown in columns 3, 4, 7, and 8 in table IB. In examining these

data a surprising result is obtained, namely, the plants from which the mother tubers were amputated gave larger yields than those from which they were not removed.

Thus (columns 3 and 4, table IB) the yield from plants from which the mother tubers were removed at stage 3 (half-grown) was 181 gm. per plant, while the yield from plants from which they were not removed was 153 gm. There were thirty-nine pairs of plants available for this comparison. When the data are treated by the method of ENGLEDOW and YULE (7), it is found that the odds are 36 to 1 that the observed difference is significant. When use is made of the formula of FISHER (8), the value of "t" resulting from the calculation is found to be 2.42. When this value is fitted into FISHER's table of "t" values it is found that his requirement of a probability at least equal to $P=0.05$ has been satisfied, and that odds greater than 19 to 1 in favor of the view that the amputated series has given greater yields than the checks have been obtained.

A probable cause of this result may be found in the fact that, in the period following stage 3, the Bliss Triumph mother tubers that remained in contact with the sprout began to rot badly. When the time arrived to amputate them at stage 4, most of the plants had to be discarded because, upon inverting the pots, it was found that the mother tubers were either partly or completely rotten. Therefore, only thirty plants with firm mother tubers were available for the comparison at stage 4. The results of the amputation at stage 4 are shown in table IB, columns 7 and 8. Here again a greater average yield was found for the plants from which mother tubers were removed, that is, 162 for the amputated and 140 for the corresponding checks. STUDENT'S (19) method shows odds of 19.5 to 1 that this difference is significant, but the calculation by FISHER'S method gives a "t" value for the observed data amounting to 1.84, which is somewhat below the "t" value (about 2.0) that would be required by him for a probability of $P=0.05$, that is, 19 to 1. Thus, while the data partially support the view that contact with the mother tuber at this stage of development has reduced the yield of tubers, the odds are not sufficient to give assurance. The evidence at stage 4, however, tends to substantiate the similar condition found at stage 3.

It will be noted in tables IA and IB, that the check plants from which mother tubers were not removed gave yields that differed considerably in the four series. Thus the checks for the amputation at stage 1 gave an average yield for Irish Cobbler of 255 gm. (table IA, column 1); the checks for stage 2 yielded 328 gm. per plant (table IA, column 5); while for stages 3 and 4 the yields of check plants were 189 and 160 gm. respectively (table IB, columns 1 and 5). Since these were check plants (mother tubers not removed), amputation could not have been a factor, yet the differences in yield were even greater than the differences due to amputation. It should be remembered that the check plants grew in pots for different lengths of time in the four series, and that after amputation the plants were of necessity transplanted into different parts of the field in the four series. Consequently the conditions for growth were considerably different, and a difference in yield could be expected. These differences between the checks in the different series, however, do not vitiate the differences between the amputated and check plants in each series, since the experiment was arranged to make conditions within each series the same for both amputated and check, except for presence and absence of mother tubers. The high variability in yield of individual potato plants, and the differences observed between checks in the four series, emphasize the importance of the precautions taken to arrange the experiment so that the amputated plants and their corresponding checks could be compared directly with each other, under conditions that make these disturbing factors inoperative.

Chemical composition of amputated mother tubers

Analyses of the tissue obtained in amputating mother tubers at intervals after planting were obtained for all three years. Although, as previously described, the yield data were not suitable for comparisons in the 1926 and 1927 experiments, because of inadequate checks or interference with the planting plan after amputation, the plants from which mother tubers were removed had made apparently a normal growth up to the time of amputation, and consequently the analytical results from the amputated tissue should be of value, and are included.

SAMPLING METHODS.—As soon as the potato tissue was removed from the sprout the pieces were wiped with a moist rag, and peeled to remove the old epidermis and the suberized layer that had formed at the cut surface. The tissue was then either minced in a food grinder or chopped in a bowl. In the preliminary experiments in 1926 and 1927 it was found that, as the season progressed, the mother tubers became very high in water content; hence when the tissue was passed through a food grinder liquid was pressed out, and it was difficult to get a sample of the tissue which represented uniform proportions of solid and liquid. In the 1928 experiments, therefore, the potato tissue was chopped into fine pieces in a wooden bowl, and better sampling obtained by this method. As soon as the tissue was ready for sampling, weighed portions were removed for the moisture determination. To obtain a sample for the subsequent analyses for all constituents except sugar, weighed amounts (150–300 gm. per sample) were dropped (small quantities at a time) into boiling alcohol. The amount of alcohol used was adjusted so that the final concentration, taking into account the water content of the tissue, would be about 70 per cent. The tissue was stored in this concentration, but when the extractions for the analyses were begun, water was added to make the alcoholic concentration 50 per cent by volume. Three extractions at boiling temperatures were made, the liquid being decanted after settling. The tissue was then dried and ground to a fine powder which was extracted twice in the same manner, the decanted portions being added to the liquid obtained by the first three extractions.

Fifty per cent alcohol (by volume) was selected as the solvent for two purposes: first, to separate the starch from non-starch substances, as recommended by BRYAN, GIVEN, and STRAUGHN (4); and second, to separate protein from non-protein nitrogen. Working with the alfalfa plant, OSBORNE, WAKEMAN, and LEAVENWORTH (13) found that alcohol at 53 per cent (by weight) precipitated protein completely from the juice, and that 20 per cent (by weight) caused the precipitation of most of the protein present. In the present experiments it was found that very little if any protein was extracted by alcohol at a concentration of 50 per cent (by volume). Tests by such protein precipitants as colloidal iron, trichloracetic acid, and

tannin produced small precipitates in which only traces of nitrogen were present. Lead acetate precipitated appreciable quantities of nitrogen, but the results of VICKERY and VINSON (22) indicate the possibility that these were substances other than protein.

The extractions were made up to a definite volume after cooling, and this liquid was centrifuged, decanted, and aliquots taken for analysis. This was called the soluble portion, solubility in this case referring to 50 per cent alcohol (by volume) as the solvent.

The small amount of precipitate obtained in centrifuging the extract was added to residue from the extraction, and this, which was called the insoluble portion, was dried on a water bath and ground to a fine powder.

Separate samples of the fresh tissue were taken for the sugar determinations, and this tissue was also dropped into boiling alcohol, exactly as just described for the main sample except that calcium carbonate was added to the alcohol.

METHODS OF ANALYSIS.—For reducing sugars the MUNSON and WALKER method (2) was used, and the precipitated cuprous oxide was titrated with potassium permanganate, the permanganate value being determined by comparison with a sample of dextrose obtained from the Bureau of Standards. Sucrose was determined by inverting in the cold with hydrochloric acid (2). Ammonia was estimated by the aeration method after adjusting the solution to slight alkalinity. The apparatus of VAN SLYKE and CULLEN (21) was used and the aeration period was 2.5 hours. For amide, the liquid after ammonia aeration was neutralized with a drop or two of acid, and, after the addition of 1 cc. of concentrated hydrochloric acid for each 10 cc. of liquid, was hydrolyzed for 1.5 hours; the liquid was then transferred to an evaporating dish and the hydrochloric acid removed by evaporation. The liquid was then made slightly alkaline and again aerated in the VAN SLYKE-CULLEN apparatus, and the ammonia formed during the hydrolysis was estimated. For "basic" nitrogen, the liquid remaining after the amide aeration was used; phosphotungstic and sulphuric acids were added, and the liquid was heated to boiling. It was then cooled, made up to volume in a flask, mixed thoroughly, and allowed to stand over night. The precipitate was separated by the use of the centrifuge; to it were added the particles of precipitate that remained in the volumetric flask, and the com-

bined precipitates were washed twice with dilute phosphotungstic acid solution. The final precipitate was analyzed for nitrogen by the KJELDAHL method (2). The liquid decanted from the phosphotungstic precipitate was used for both the non-basic and amino-acid nitrogen, the non-basic by the KJELDAHL method, and the amino-acid gasometrically by the VAN SLYKE procedure (20). For the estimation of starch the diastase method with subsequent acid hydrolysis was used (2), but in this case saliva was found preferable to the malt extract because of the low "blank" value with saliva.

RESULTS OF ANALYSES.—The percentage composition calculated on the basis of fresh weight of the tissue is shown in table II and fig. 5. It will be noted that a rapid fall occurred in the percentages of all constituents except moisture, sugars, and soluble solids. Thus while the starch percentage dropped from about 10–15 to about 0.3–0.6 per cent (table II, column 8), the water content increased from about 70–80 to about 96 per cent. The various forms of nitrogen (table II, columns 11–17) all show a continuous reduction in percentage composition throughout the period of analysis, until finally the mother tubers removed at stage 4 were almost completely depleted of all forms of nitrogen, the percentage remaining being only about one-fifth or one-tenth of that present in the mother tubers at the time of planting. The change in the percentage of reducing sugar shows an interesting behavior in that, although there is a continuous loss of dry substance, the percentage of reducing sugar actually increased (table II, column 9). The details of these changes vary somewhat with the variety and with different years. There is a tendency for the percentage to be highest in midseason, that is, when the plants are about 10 inches high, and to recede again at the later stages. Another interesting feature is to be noted with respect to the percentage of solids soluble in 50 per cent alcohol. This tends to remain more nearly constant than any other constituent for which data were obtained. Thus, up to about stage 3 (stage 2 in the case of Bliss Triumph in 1928) the percentage of soluble solids was about 80 per cent of the value at the start of the experiment. Probably the increase in sugar explains this maintenance of a high soluble solids value in spite of the continuous loss of dry weight during the same period. Later in the season the percentage of soluble solids

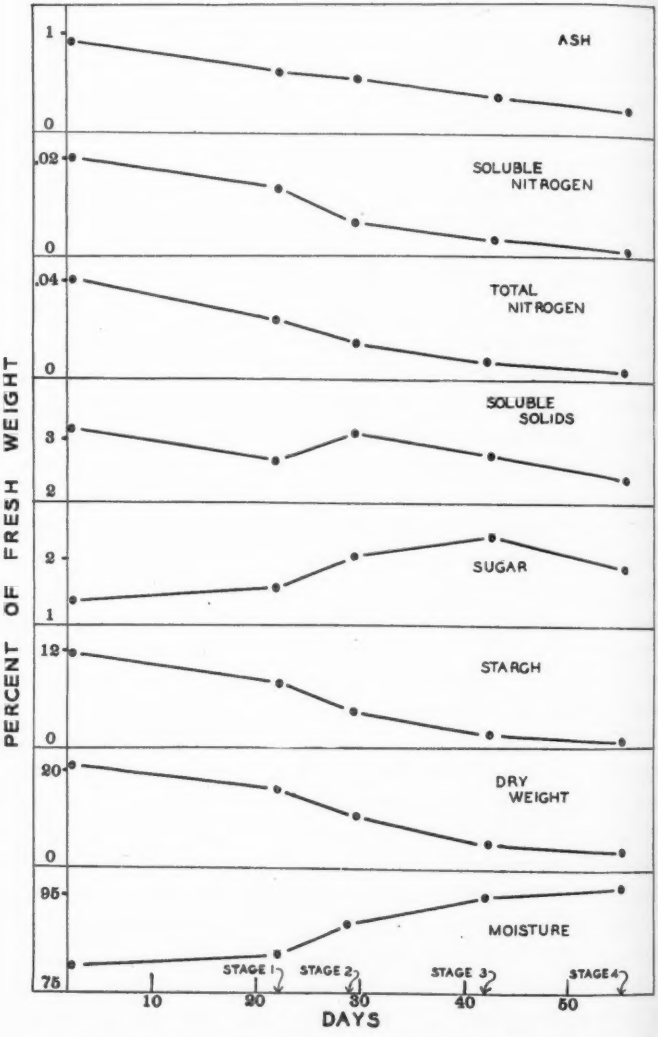


FIG. 5.—Composition of mother tuber tissue at beginning of experiment and at amputation at different stages of growth: points in circles over abscissa at 22 days show composition of mother tuber tissue removed at stage 1, those over the 29-day abscissa at stage 2, etc.; ordinates show percentages on fresh weight basis. Note change in percentage range for each constituent (left) to correspond to requirements of different constituents.

TABLE II

CHEMICAL COMPOSITION OF MOTHER TUBERS REMOVED FROM POTATO SPROUTS AT DIFFERENT INTERVALS AFTER PLANTING

VARIETY AND DATE OF PLANTING	DATA REGARDING REMOVAL OF MOTHER TUBERS				PERCENTAGE COMPOSITION ON FRESH WEIGHT BASIS												
										Nitrogen (N)							
	Date of removal	No. days after planting	No. days after emergence of sprout	Condition of plant at time of removal	H_2O	Solids soluble in 50% alcohol	Starch	Reducing sugars	Cane sugar	Insoluble in 50% alcohol	Soluble in 50% alcohol	NH_3 N	Amide N	Basic N	Non-basic N	Amino N
Irish Cobbler, May 12, 1926	Seed-pieces at start, before planting				71.6	2.80	15.72	0.55	0.15	0.17	0.40	0.023	0.014	0.087	.....	0.078
	June 4	23	2	.....	87.6	2.51	6.15	0.99	0.11	0.06	0.27	0.027	0.009	0.057	.....	0.051
	June 14	33	12	.....	91.8	2.62	3.05	1.88	0.08	0.04	0.20	0.012	0.010	0.033	.....	0.044
	June 21	40	19	.....	94.3	2.41	1.41	1.93	0.12	0.03	0.11	0.011	0.005	0.026	.....	0.024
	June 29	48	27	.....	96.0	2.11	0.48	1.61	0.15	0.02	0.08	0.013	0.002	0.021	.....	0.027
	July 7	56	35	.....	95.5	1.94	0.32	1.48	.....	0.03	0.06	0.007	0.001	0.012	.....	0.014
Irish Cobbler, April 22, 1927	Seed-pieces at start, before planting				80.4	2.81	10.61	0.50	0.16	0.13	0.21	0.017	0.023	0.076	.....	0.096
	May 28	36	7	Sprouts 2" high	89.8	2.35	3.82	1.28	0.50	0.08	0.07	0.007	0.010	0.027	.....	0.039
	June 16	55	26	Sprouts 9" high	95.3	2.04	0.28	1.42	0.24	0.04	0.03	0.002	0.005	0.019	.....	0.015
	June 30	69	40	Sprouts 13" high	96.3	1.70	0.60	1.43	0.07	0.03	0.01	0.001	0.002	0.025	.....	0.009
Irish Cobbler, May 2, 1928	Seed-pieces at start, before planting				78.90	3.17	11.80	0.89	0.46	0.21	0.20	0.008	0.036	0.040	0.100	0.084
	May 24	22	0	Emergence of sprouts (stage 1, fig. 2A)	83.70	2.93	8.15	1.20	0.40	0.11	0.15	0.005	0.019	0.050	0.060	0.047
	May 31	29	7	About 2" high, leaves expanded (stage 2, fig. 2B)	88.60	3.15	4.89	1.42	0.72	0.07	0.08	0.005	0.009	0.030	0.035	0.025
	June 11	42	20	About 10" above ground, half-grown (stage 3, fig. 2C)	94.40	2.82	1.09	2.02	0.42	0.03	0.04	0.002	0.006	0.014	0.014	0.015
	June 25	56	34	Maximum height, in bloom (stage 4, fig. 2D)	96.00	2.27	0.30	1.61	0.21	0.02	0.02	0.001	0.003	0.007	0.006	0.008
Bliss Triumph, May 2, 1928	Seed-pieces at start, before planting				81.72	3.66	9.55	1.11	0.63	0.11	0.17	0.007	0.026	0.042	0.071	0.054
	May 24	22	0	Emergence of sprouts (stage 1, fig. 2A)	85.25	2.87	7.93	1.24	0.29	0.10	0.11	0.004	0.014	0.030	0.044	0.028
	May 31	29	7	About 2" high, leaves expanded (stage 2, fig. 2B)	91.90	3.35	3.33	1.86	0.46	0.06	0.05	0.002	0.007	0.013	0.018	0.014
	June 11	42	20	About 10" above ground, half-grown (stage 3, fig. 2C)	96.40	2.22	0.40	1.40	0.49	0.03	0.02	0.002	0.003	0.007	0.007	0.007

dropped, but even at stage 4 its value was found to be 60–70 per cent of the original value.

The ash analyses, given in table III, are for the 1928 series only. Columns 2 and 5 show the percentage of ash obtained by ashing the portion of the tissue that was insoluble in 50 per cent alcohol; columns 3 and 6 show the ash percentages obtained by ashing aliquots of the liquid extract; and columns 4 and 7 represent the total ash obtained by adding the soluble and insoluble percentages. These analyses show a gradual fall in the different forms and in the total ash from the beginning to the end of the experiment. The ash,

TABLE III

ASH IN MOTHER TUBER TISSUE AMPUTATED FROM SPROUTS AT DIFFERENT PERIODS OF GROWTH

STAGE OF GROWTH AT TIME OF AMPUTATION	PERCENTAGE ON FRESH WEIGHT BASIS					
	IRISH COBBLER			BLISS TRIUMPH		
	Insoluble*	Soluble*	Total	Insoluble*	Soluble*	Total
Start of experiment.....	0.425	0.455	0.880	0.285	0.316	0.601
Stage 1 (fig. 2A).....	0.337	0.368	0.705	0.273	0.300	0.573
Stage 2 (fig. 2B).....	0.302	0.310	0.612	0.195	0.197	0.392
Stage 3 (fig. 2C).....	0.208	6.195	0.403	0.167	0.128	0.295
Stage 4 (fig. 2D).....	0.020	0.160	0.180	†	†	†

* Percentage ash in the portion of tissue either soluble or not soluble in 50 per cent alcohol (by volume).
† Tissue not available for analysis (see text).

however, was not used up as completely as the dry weight or various forms of nitrogen, as shown in the previous paragraphs. Table III shows that at stage 3 approximately one-half of the ash was still present in the mother tissue. An analysis of the ash for various constituents was not made, but the ash has been retained for a subsequent analysis. The question as to the rate of utilization of the different ions is an interesting one, made especially so by the observations of MÜLLER (12) and of RAMSEY and ROBERTSON (14). In both contributions it is reported that potassium remains in the mother tuber and does not take part in the growth of the sprout.

CHANGES IN FRESH AND DRY WEIGHT OF INDIVIDUAL MOTHER TUBERS.—Since the seed-piece loses dry substance to the sprout, and also of course loses substance by its own respiration, and at the same

time gains in water content, it may be inquired whether, as a result of these two opposing factors, the absolute weight of the mother tuber will increase or decrease during the period of the experiment.

In the 1926 and 1927 experiments, no account was taken of the absolute change in weight of the mother tuber during the season. Hence, although the data could be calculated upon either the fresh or dry weight basis, it was impossible to determine the absolute amounts of materials leaving the individual mother tuber at any stage. In 1928 a special experiment was carried out to obtain data on this point (table IV). About 75 seed-pieces of each variety were carefully weighed on a small balance in order to obtain a weight of 28 gm. Ten of these were weighed at once, and the average weight was found to be 28.20 gm. for Irish Cobbler and 28.25 gm. for Bliss Triumph (table IV). The average deviation of the weight of any one seed-piece from the average weight of the ten was about one per cent. The other sixty-five pieces of each variety were then planted in the soil, and permitted to grow until the plants arrived at the various stages of development at which mother tubers were amputated in the main experiment. At each stage ten plants were lifted, the mother tubers removed, and after careful wiping with a moist cloth, the fresh and dry weight of each was obtained. The average values are shown in table IV. The changes in fresh weight of the individual mother tubers are shown in columns 2 and 3. It is seen that the absolute weight did not change much throughout the season, the Irish Cobblers losing in weight from 28.20 to 26.22 gm., and the Bliss Triumph mother tubers gaining in weight from 28.25 to 29.02 gm. The dry weight and water content per mother tuber are expressed in grams in columns 4, 5, 6, and 7 in table IV. Thus, while each Irish Cobbler seed-piece lost 5.24 gm. of dry matter (that is, from 6.43 to 1.19 gm.), it gained 3.26 gm. of water (that is, from 21.77 to 25.03 gm.). This observation, that the absolute weight of the mother tuber did not change to any great degree during the season, facilitated the interpretation of the data obtained from the samples of tissue. It showed that calculations upon the fresh weight basis are capable of showing the true changes in composition with respect to the various constituents. And, although the observations showing that this is true were not made until 1928, it seems likely

that the 1926 and 1927 data can be evaluated safely from the same point of view.

In the last six columns in table IV the changes in fresh weight, dry weight, and water content of the mother tubers at each stage of development have been calculated from these data, and expressed as a percentage of the amount in the mother tuber at the time of planting. Special attention is directed to the large percentage losses in dry substance. About 25–30 per cent was lost by the time the sprout emerged from the soil, about 50 per cent at the time germination was complete, and about 80 per cent at the time the plant was half-grown. It is not maintained, of course, that all the dry substance lost by the mother tuber was gained by the sprout. It is certain that part of it was lost by the respiration of the mother tuber tissue, and some of it might merely have leached out into the soil.

Absolute amounts of various chemical constituents per mother tuber

The data in table IV, showing the average fresh weight per mother tuber at each stage of development, combined with the data in table II, showing the percentage composition on the fresh weight basis, permitted a calculation of the absolute amounts of the various constituents in the individual mother tuber at each stage at which amputation was carried out. These data are shown for the 1928 experiments in table V.

By following down the columns in table V it is possible to observe the amounts of the various constituents present at the time of planting, and also the amount remaining in the mother tuber at each subsequent period of development.

One of the interesting features of table V relates to the utilization of starch between stages 2 and 3. At stage 2 leaves were fully formed, and the production of starch by the process of photosynthesis was going forward rapidly. It might have been expected that because of this there should have been less demand upon the stored starch, and yet during this period of high photosynthetic activity in the leaves the depletion of starch from the mother tuber was going forward at a rapid rate.

Another striking result was obtained with respect to the utiliza-

TABLE IV

FRESH WEIGHT, DRY SUBSTANCE, AND WATER CONTENT OF MOTHER TUBERS REMOVED FROM SPROUTS AT INTERVALS AFTER PLANTING

DESCRIPTION OF MOTHER TUBERS REMOVED FROM SPROUT	FRESH WEIGHT PER MOTHER TUBER (GM.)		DRY WEIGHT PER MOTHER TUBER (GM.)		WEIGHT OF H_2O PER MOTHER TUBER (GM.)		PERCENTAGE OF AMOUNT IN MOTHER TUBER AT TIME OF PLANTING					
							Fresh weight		Dry weight		Water	
	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss
As planted	28.20	28.25	6.43	5.47	21.77	22.78	100	100	100	100	100	100
Emergence of sprout (stage 1, fig. 2A)	27.40	28.88	4.45	4.17	22.96	24.71	97	102	69	76	105	109
After germination complete and leaves expanded (stage 2, fig. 2B)	27.46	29.24	3.20	2.79	24.26	26.45	97	104	50	50	111	116
Plants 10 inches high and young tubers starting to form (stage 3, fig. 2C)	26.30	29.02	1.31	1.08	24.99	27.93	93	103	20	20	115	122
Plants in bloom and young tubers well developed (stage 4, fig. 2D)	26.22	*	1.19	*	25.03	*	93	*	19	*	115	.....

* Because of rotting of mother tubers of Bliss Triumph samples, a firm condition could not be obtained at this stage of development.

TABLE V

ABSOLUTE AMOUNTS OF SUBSTANCES PER MOTHER TUBER AFTER REMOVAL FROM SPROUTS AT INTERVALS AFTER PLANTING

CONDITION OF MOTHER TUBERS REMOVED FROM SPROUT	WATER (GM.)		SOLIDS SOLUBLE IN 50% ALCOHOL (GM.)		STARCH (GM.)		REDUCING SUGARS (GM.)		CANE SUGAR (GM.)		NITROGEN (N)													
											Insoluble in 50% alcohol (gm.)		Soluble in 50% alcohol (gm.)		NH_3 N (gm.)		Amide N (gm.)		Basic N (gm.)		Non-basic N (gm.)		Amino N (gm.)	
	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss
As planted	21.771	22.779	0.894	1.034	3.328	2.698	0.251	0.314	0.130	0.178	0.059	0.031	0.056	0.048	0.0023	0.0019	0.0102	0.0074	0.0113	0.0119	0.0282	0.0201	0.0237	0.0153
Emergence of sprout (stage 1, fig. 2A)	22.958	24.717	0.803	0.835	2.233	2.290	0.329	0.358	0.110	0.084	0.030	0.029	0.041	0.032	0.0014	0.0012	0.0052	0.0041	0.0135	0.0086	0.0164	0.0127	0.0129	0.0081
After complete germination and leaves expanded (stage 2, fig. 2B)	24.256	26.450	0.865	0.981	1.343	0.974	0.390	0.544	0.198	0.135	0.019	0.017	0.022	0.014	0.0014	0.0006	0.0025	0.0020	0.0082	0.0038	0.0096	0.0052	0.0069	0.0040
Plants 10 inches high and young tubers starting to form (stage 3, fig. 2C)	24.990	27.935	0.742	0.644	0.287	0.116	0.531	0.406	0.110	0.116	0.008	0.009	0.011	0.006	0.0005	0.0006	0.0016	0.0008	0.0037	0.0020	0.0037	0.0020	0.0039	0.0020
Plants in bloom and young tubers well developed (stage 4, fig. 2D)	25.029	*	0.567	.....	0.079	.....	0.402	.....	0.055	.....	0.005	.....	0.005	.....	0.0002	.....	0.0006	.....	0.0018	.....	0.0015	.....	0.0021	.....

* At this stage of growth in the 1928 experiments, Bliss Triumph mother tubers were so badly rotted that a sufficient number could not be recovered.

tion of the sugars. Since the sugars are physiologically quite active, and therefore in condition for immediate use in respiration and growth, it might be expected that during periods of rapid growth these mobile substances would be used up rapidly and their concentration reduced to the minimum. Instead, however, the sugar actually increased during the period of rapid growth (see footnote 2). No doubt it was transported freely from the mother tuber, but this did not result in depletion, since apparently other compounds were broken down to sugar, and a high sugar level was maintained. The cane sugar situation is similar to that with reducing sugar, but the rate of loss of cane sugar was greater in the later stages of development (table V, columns 10 and 11).

The rate of disappearance of nitrogenous substances from the mother tubers is shown in table V, columns 12–25. The significant feature here is that all the forms of nitrogen for which analyses were made were utilized at about the same rate. It might reasonably have been expected that some forms would disappear faster than others, but the uniformity of the disappearance of these diverse groups is surprising. An estimate of this may be obtained by noting the number of cases in which the amount remaining at any stage is about one-half of that remaining at the previous stage. Out of forty-nine such pairs of values, thirty-one may be classed as essentially fulfilling this condition. Nothing of fundamental importance is claimed for this relation, which is quite accidental, since the stages chosen for amputation were merely empirical, but the coincidence serves to emphasize the facts observed. The data for 1926 and 1927 in table II also show a somewhat similar condition with respect to the rate of utilization of nitrogenous reserves.

BEHAVIOR OF DIFFERENT VARIETIES WITH RESPECT TO LOSS OF DRY WEIGHT

Because of the discovery of SELIBER (17) that the substance of the mother tuber of the variety Kruger is not utilized to any considerable extent by the sprout, a test was made with several varieties in order to see whether this behavior could be observed with American varieties. Tubers of ten varieties,[4] in addition to the Irish Cob-

[4] Appreciation is hereby expressed for the co-operation of Mr. WILLIAM STUART of the United States Department of Agriculture in supplying tubers of these varieties (except King Edward) for this experiment.

blers and Bliss Triumph used in the main experiment, were available for this test. Seed-pieces were prepared by first adjusting the fresh weights at approximately 20 gm. each, and then weighing them accurately on a balance. The average fresh weights are shown in table VI, column 2, and the corresponding dry weights in column 4. The seed-pieces were then planted and allowed to grow until the plants were about half-grown. The plants were then lifted, the mother tubers wiped carefully with a moist cloth, and the fresh and dry

TABLE VI

COMPARISON OF DIFFERENT VARIETIES OF POTATOES IN LOSS OF DRY WEIGHT FROM MOTHER TUBERS

VARIETY	FRESH WEIGHT OF MOTHER TUBER (GM.)		DRY WEIGHT OF MOTHER TUBER (GM.)		PERCENTAGE DRY WEIGHT LOST
	At planting	After amputation from sprout*	At planting	After amputation from sprout*	
Early Rose	20.34	22.56	4.760	1.334	72
King Edward	20.33	24.07	3.923	0.956	76
Green Mountain	20.37	20.22	5.213	1.333	74
Burbank	20.19	19.37	4.656	1.080	77
Russet Rural	20.06	25.23	4.339	1.137	74
Peerless Pearl	20.66	21.69	4.741	0.696	85
Beauty of Hebron	19.60	22.74	4.543	1.286	72
Early Ohio	20.03	19.10	4.671	0.742	84
Ehnola	20.07	20.57	5.260	0.810	85
McCormick	20.85	21.49	3.621	0.859	76

* Amputation from sprouts after germination had been completed and plants were about 15 inches high.

weights of each again determined (table VI, columns 3 and 5). The right-hand column in table VI shows the percentage of the original dry weight lost by the mother tubers during the period of growth. It is apparent that none of the varieties showed a behavior similar to that of Kruger. All the varieties in the present test lost a large proportion of their dry weight. Early Rose and Beauty of Hebron lost 72 per cent of their original dry weight, and Peerless Pearl and Ehnola lost 85 per cent.

It is interesting to note also in this table the data available on the change in fresh weight of mother tubers during the season. In the previous discussion of the behavior of Irish Cobbler and Bliss Triumph, it was pointed out that only small changes in the weight

of the mother tuber took place, Irish Cobbler losing slightly in weight and Bliss Triumph gaining slightly. Table VI, columns 2 and 3, shows that of the varieties in this test, Green Mountain, Burbank, Peerless Pearl, Early Ohio, Ehnola, and McCormick showed such small changes that it may be doubted whether the amount of change is greater than the errors of measurement. Relatively large increases in fresh weight were shown by King Edward and Russet Rural. SELIBER (17) has emphasized the tendency of the tubers to gain in weight during the season, but in the present experiments the more usual tendency has been for the fresh weight to remain nearly constant. Probably the condition of the seed-piece at the time of planting and the weather conditions during growth are important factors.

Discussion

It should be emphasized that the results of this series of experiments were obtained with mother tubers 28 gm. in weight. This size was selected because from the literature it appeared that this is about the smallest size that can be depended upon to produce a plant with full vigor. And while in these experiments removal of mother tubers at midseason or later did not markedly reduce the final yield, it is possible that with smaller seed-pieces the influence of the mother tuber would have been exerted during the later periods of growth. Likewise, although a reduction in yield resulted from the removal of mother tubers after germination was complete and leaves were fully formed, it is possible that if large seed-pieces had been used, and if large supplies of stored materials had been available during the early stages of germination, the young plant might have become independent of the mother tuber at an earlier stage. Further work regarding the relation of size to the stage of development at which contact with the seed-piece is no longer critical for growth of the sprout would be desirable.

It may have been noted that the analytical data are presented on the fresh weight and the "per tuber" basis, but not on the dry weight basis. The results of the analyses in these experiments are peculiarly well fitted to emphasize the importance of the selection of the basis on which chemical analyses are calculated, and that erroneous conclusions may result from the use of a basis that is un-

suitable for the purpose. It is clear that in the present case the "per tuber" basis is the best one to show the rate of utilization of the stored substances, since in this way we deal with absolute amounts. The fresh weight basis is nearly as good, since in this particular case the fresh weight of a seed-piece is nearly constant throughout the period of sampling. If the information in table V (showing the absolute amounts of various constituents per mother tuber), and that in table IV (showing the dry weight per mother tuber) is used to calculate the percentage composition on the dry weight basis, some very misleading results are obtained. This method of calculation shows, for example, that at stage 2 the starch percentage of the Irish Cobblers was about 19 per cent lower than at the beginning, when as a matter of fact about 60 per cent of the starch had been lost by that time. Again, this method of calculation shows that little change in amino acid content had taken place between the beginning of the experiment and stage 3; but the mother tuber had lost, in fact, 83 per cent of this constituent. This confusion results from the condition that as the substances are used up the dry weight itself also falls, and the percentage change on this basis depends upon the relative rate of fall of the two changing values.

The observations (table IB, columns 1 and 2) that in midseason it appears possible that the Irish Cobbler plants had become completely independent of the mother tubers, and that amputation may not have influenced the yield unfavorably, are not in accord with the observations of SIKORSKI (18) and SELIBER (17). It must be remembered, however, that they suggest the importance of the mother tuber as an organ for water storage, and this factor would come into play only in periods of drought. In the present experiments there was an abundance of moisture in the soil at all times, and therefore no opportunity was afforded to test their hypothesis. The fact that in the preliminary experiments removal of the mother tuber by cutting across the base of the stem with a knife, or amputating by twisting, induced wilting of the plant even under moist conditions, lends support to the view that the water stored in the mother tuber might become an important factor.

The unfavorable effect upon the yield of Bliss Triumph which was obtained when the mother tubers were allowed to remain in con-

tact with the plant from stage 3 to stage 4 (table IB, columns 3 and 4) may have resulted from toxic materials formed during the rotting of the tubers. The reports of BREAZEALE (3) and COLLISON (5) emphasize the toxic effects of plant tissue during certain stages of decomposition. If toxic substances were formed during the rotting of mother tubers, the conditions for their absorption by the plant would be favorable because of the close connection at the base of the stem between the mother tuber tissue and the sap-conducting vessels.

DE VRIES (6) believed that the mother tuber furnished nutrients to the sprout until germination was complete, after which time its substance was transferred exclusively to the newly developing tubers. LUDWIG (11) adopts this as an argument against large tubers for seed-pieces, on the basis that it is not an economical method, since much of the starch in the large tubers merely passes out of the old into the young tubers, and at the end of six months is harvested a second time. There is evidence against these views in the results of the present experiments. Fig. 2 shows that at stage 2 very few young tubers had begun to form, and that even at stage 3 the new tubers were still small. The analyses (tables II and V, and fig. 5) show that at stage 3 nearly all of the starch and various forms of nitrogen had already been exhausted from the mother tuber; in fact, only 20–25 per cent of the original dry weight still remained, and the subsequent analyses showed that not much more transference from the mother tuber was to take place. Most of the materials destined to leave the mother tuber did so within about forty days from planting, or about twenty days from the date of emergence of sprout. We are not justified in inferring from this that the small amount of substance left at this stage does not exert a considerable influence upon the later growth of the plant, but it suggests that this influence is qualitative, and not due to the quantity of starch or other materials furnished.

This brings up the question as to the nature of the chemicals that pass from the mother tuber to the sprout and strongly influence its growth. Are these materials merely food stuffs, or are they special growth-promoting substances, such as have been postulated by APPLEMAN (1)? The present experiments seem competent to show that materials ordinarily regarded as food stuffs, such as sugar,

amides, amino-acids, etc., are used up rapidly and disappear from the mother tuber, and we must assume that they constitute an important part of the contribution made by the seed-piece. But the experiments are not capable of showing that special substances do not pass also, and that these do not exert an important function in regulating the use of the food stuffs that are translocated. We may regard the situation as analogous to that found by REED (15) in the twigs of lemons and other plants, in which two factors are considered to be operating jointly: first, stored food which (when in the proper condition) can support growth; second, special substances which (in small quantities) influence the utilization of these food materials in growth. REED speaks of them as substances which catalyze the growth process. In any event it is clear that the methods of analysis used in my experiments are not capable of showing the presence of special growth-promoting substances. The use of culture solutions would offer a more favorable method of experimentation; and the results of LUDWIG (11), showing the influence of salt content of the culture solution in modifying the rate of utilization of the stored foods in the tuber, are suggestive of the capacity of small amounts of one substance to control the utilization of a relatively large quantity of other substances in growth.

Summary

1. A series of experiments, in 1926, 1927, and 1928 is reported, in which the mother tubers were amputated from potato plants at intervals after planting, in order to observe the effect of this removal upon the subsequent growth and yield of the plant, and to note at what stage of development the young plants became independent of the food reserves of the seed-piece.

2. The seed-pieces were planted in the bottoms of pots which were then buried in the soil; when the sprouts had reached certain sizes the pots were removed and inverted, exposing the mother tuber, which was then amputated; the pot was discarded and the well-rooted plant replaced in the soil. The checks consisted of an equal number of plants which were removed from the pots at the same time but which were allowed to retain the mother tubers.

3. The mother tubers were removed at the following periods of

development: stage 1, when the sprouts first emerged from the soil and before leaves had expanded; stage 2, when plants were about 2 inches and had leaves fully expanded; stage 3, when plants were about 10 inches high and young tubers were forming on well-developed stolons; stage 4, when plants had attained approximately maximum height and were in bloom.

4. The effect of amputation upon subsequent growth is shown by photographs, and by tables of the yield. Plants from which the mother tubers were removed at stage 1 gave a yield of tubers only about one-third of that of the checks; removal of the mother tuber at this stage was critical and many plants were not able to survive. The plants from which mother tubers were removed at stage 2 gave a yield which was about 80 per cent of that of the corresponding checks, but the difference in yield was significant statistically, indicating that at this stage of development the young plant has not yet become independent of the stored food in the seed-piece. With respect to the effect of amputation at stage 3, the two varieties used (Irish Cobbler and Bliss Triumph) gave different responses. In the case of Irish Cobbler, plants from which mother tubers were removed showed a reduction in yield per plant, but the odds that this difference was significant were not high, leaving the issue in doubt. In the case of Bliss Triumph, the amputated plants gave a higher yield than the checks, indicating that contact with the mother tuber had been detrimental. It is suggested that this result was due to the rotting of the mother tubers between stages 3 and 4, with the production of toxic substances which were absorbed from the seed-piece at the base of the main stem and unfavorably influenced the subsequent growth. This effect was not observed in the case of Irish Cobbler seed-pieces, since they remained firm between stages 3 and 4. Removal of mother tubers at stage 4 produced no effect upon the yield of Irish Cobbler, but again the yield of Bliss Triumph was slightly greater with mother tubers off than with them on. The difference in this case, however, was not large enough to be conclusive statistically.

5. It appeared possible that the sprout became independent of the mother tuber at stage 3 (about 10 inches high). Most of the storage materials that were destined to leave the mother tuber had

already been used up. Further experiments are required to show conclusively whether contact with the mother tuber at this or later stages is beneficial; and if so whether the advantage results from the transfer of the small amount of food materials still available at that time, or from the water storage relation existing between seed-piece and sprout.

6. These experiments were carried out with mother tubers weighing 28 gm. each. It is possible that with smaller seed-pieces the sprout would have remained dependent on the mother tuber for a longer period, and that with larger pieces amputation could have been carried out at an earlier period without interfering with subsequent development.

7. The amputated mother tissue was subjected to chemical analyses in order to determine the rate at which stored substances left it, and to note any correlation which might exist between the composition at any stage of development and the subsequent behavior. A rapid loss of substance from the mother tuber was noted. By the time the sprout emerged, about one-fourth to one-third of the dry weight was lost; about one-half remained at stage 2; and at stage 3 the mother tubers had been depleted of nearly 80 per cent of the original dry weight. The extent of the depletion varied with different lots, in different years, and with different varieties, but ranged from 70 to 85 per cent. Starch and the various forms of nitrogen (both soluble and insoluble) were steadily used up; but the sugar concentration was maintained at a high level, so that, even though the dry weight had been reduced to a low level the sugar percentage on the fresh weight basis was greater at all subsequent stages than it had been at the time of planting.

8. The analyses showed that different groups of nitrogenous substances, such as insoluble, soluble, ammonia, amide, amino, basic, etc., were removed from the mother tuber at approximately the same rate. There was no evidence of one form being more readily available for growth than other forms.

9. Although between stages 2 and 3 the foliage was active photosynthetically, large demands upon the organic substance of the mother tuber were made during this period. A "sparing" action

upon stored foods in the underground part because of food manufacture in the tops of the plant was not noted.

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GERMINATION AND KEEPING QUALITY OF PARSNIP SEEDS UNDER VARIOUS CONDITIONS[1]

HILDA C. JOSEPH

(WITH TWO FIGURES)

Introduction

In several ways parsnip seeds furnish especially favorable material for the study of the effect of storage conditions upon longevity. They are short-lived, and when stored in bags in a laboratory the vitality drops about 20 per cent during two years and 60 per cent during three years of storage; hence the differential effect of storage conditions is quite evident within two years. They withstand extreme drying without injury to their vitality, which makes it possible to study the effect of a wide range of water content. They germinate with reasonable speed over a considerable range of temperature.

The experiments reported in this paper have been conducted for the purpose of finding a method by which parsnip seeds could be stored for several years without losing their vitality. Such a method would be of advantage to seedsmen in enabling them to avoid an annual discard of seeds left over from the previous year. It is also hoped that a method worked out for storage of parsnips might be applicable with modifications to other short-lived seeds of commercial importance, such as cabbage, onion, and a number of the coniferous seeds.

The problems which presented themselves in connection with this study of parsnip seeds were mainly the following: (1) Is the seed material as harvested and sold by the seed grower uniform enough for experimentation, or do the ripe and green seeds of each shipment require separate investigation? (2) What are the optimum temperature requirements for the germination of parsnip seeds, and does the optimum germination temperature remain unchanged when the seeds become older? (3) What influence have moisture content, stor-

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age temperature, humidity of storage chamber, fluctuation of humidity, and aeration upon the keeping quality of the seeds? (4) In which way or ways do the factors mentioned under (3) influence the viability of parsnip seeds?

Discussion of literature

A number of investigations have been made to determine the influence of various storage conditions upon the keeping quality of seeds. This discussion will be limited, however, to the reports of DUVEL (1), HEINRICH (2), MAQUENNE (3), NAKAJIMA (4), and TILLOTSON (5), which bear directly on the work reported in this paper.

The paper of MAQUENNE is the only one in which experiments with parsnip seeds are reported. MAQUENNE stored parsnips in vacuum for two years at room temperature. During this period the seeds retained their viability completely, while a control lot stored in a paper bag lost all vitality during the same period. Excessive drying, according to MAQUENNE, is the only way of successfully retaining viability in certain seeds, because only when the last traces of water are liberated from the seed under the action of the vacuum is a state of "supermaturation" reached. He defines supermaturation as "a new state of equilibrium established between the enzymes and the substances they condense," and speaks of it as a state of suspended life.

Since MAQUENNE's experiments ran only a little more than two years (1899–1902), it remains to be proved whether he really was able to produce a state of suspended life in his experimental material, or whether the life processes were only retarded enough to leave the final percentage of germination unchanged for a certain time. He does not give data to show whether the germination energy as well as the germination power remained constant. He also fails to show that there is no other way of storing by which vitality could be maintained in short-lived seeds. Since the data given are based on quantities of 2–4 gm. of seeds which were originally not of a very high quality (51 per cent of vital seeds at the beginning of the experiment), it seemed desirable to investigate the keeping quality of a larger number of parsnip seeds of good quality under a variety of storage conditions.

DUVEL (1) worked with a number of vegetable seeds, which he

stored under various climatic conditions in different places of the United States, such as Mobile, Alabama; Wagoner, Indian Territory; and Ann Arbor, Michigan. He determined the effect of various degrees of atmospheric humidity and temperature on the keeping quality of his material, and worked out different methods of packing and sealing seeds as a protection against the influence of high atmospheric humidities. One of his main conclusions is that "as a factor detrimental to vitality, moisture is of far greater importance than temperature."

DUVEL was working with atmospheres of high constant humidity and widely fluctuating humidities without distinguishing between the effects of the two. Later TOUMEY (6) emphasized the detrimental effect of variations in humidity, even when the moisture content reached at different times was not very high, making the following statement:

> Even the most resistant species suffer . . . . when kept over summer in a loft where the moisture content of the seed is likely to vary with variations in the humidity of the air.

This quotation applies to forest seeds, especially to those of the coniferous type, and is taken from the chapter on seed storage, which contains very good general information as to optimum storage conditions for various tree seeds.

HEINRICH (2) has made significant contributions to our knowledge of the effects of storage conditions upon the vitality of seeds of cereal and forage crops. He determined the rate at which these seeds absorb water from a saturated atmosphere, also the total amount thus absorbed. He also found the "critical moisture content" for seed storage. With moisture content above the critical point there is rapid degeneration of the seeds in storage, while with moisture content below it the vitality is little modified by a considerable period of storage. Discovery of the critical moisture content is one of the very important contributions to our knowledge of seed storage. It is also a fact that has been largely overlooked by later investigators.

TILLOTSON (5) shows the favorable influence of dry air-tight storage upon the retention of vitality by coniferous seeds. His method avoids the injurious effects of fluctuating moisture content. He did not determine the exact moisture content of the seeds, but the

excellent keeping quality shows that they were below HEINRICH's critical moisture content.

NAKAJIMA's experiments (4), published in 1927, are interesting because of the methods used for drying the seeds. In addition to drying over desiccating agents, he mixed quicklime or calcium chloride with the seeds, and varied the amount of the drying agent added to suit the amount of desiccation endured by the various seeds, thus avoiding excessive or injurious drying. He considers his method of controlled drying in closed chambers superior to "air-dry" air-tight storage.

Methods and material

In these experiments one variety of parsnip, commercially called Hollow Crown, was used exclusively. The supply was obtained from two different commercial seed firms in three shipments in the fall of 1924, and from one firm in one shipment in the fall of 1925. The shipments of 1924 (no. 24266, no. 24702, and Hollow Crown Guernsey) were lacking in uniformity, and were therefore separated into ripe and green lots. The shipment of 1925 appeared to be uniform enough to remain unselected. The total number of seeds handled in all experiments approximated 85,000.

All germination tests were carried out in electrically heated ovens, the temperature of which was automatically regulated. The seeds were placed on three thicknesses of filter paper in Petri dishes, in lots of 100 seeds in each dish. The seeds were left unsterilized after it had been determined that the loss, resulting from treatment with various concentrations of uspulun, was more severe than that caused by molding of the seeds. Alternations of temperature were effected by transferring the seeds from one oven to another, with the longer period (5:00 P.M.–9:00 A.M.) at the lower temperature.

The seeds were stored in glass bottles with rubber stoppers. When seeds with reduced moisture content were stored, the stoppers were cemented in the bottles and coated with DeKotinsky cement. Either the germination chambers just mentioned or an ice chest served as storage chamber. In each chamber the seeds remained in darkness except for the short intervals when the doors were opened. The moisture determinations were made on unmacerated seeds in ovens at 103° C., and in a vacuum oven at 72° C.

Experimental results

Before storage experiments were begun, preliminary studies were made with the different samples of Hollow Crown seeds: (1) to determine the vitality and optimum germination temperature of fresh seeds; (2) to determine the moisture content of air-dry green and ripe seeds; (3) to improve the germination of green seeds by artificial drying.

The vitality of ripe seeds (brown seed coats) proved to be superior to that of seeds not fully ripe (green seed coats). There is no specific optimum germination temperature for ripe or green seeds of recent harvest. As table I shows, they germinate equally well at

TABLE I

GERMINATION OF FRESHLY HARVESTED PARSNIP SEEDS; COMPLETE GERMINATION REQUIRED 21 DAYS IN EACH CASE

COLLECTION (1924)	CONDITION OF SEED	NO. OF SEEDS IN EACH TEST	PERCENTAGE GERMINATION AT					
			15° C.	20° C.	27° C.	32° C.	15–20° C.	20–32° C.
Hollow Crown no. 24702.	Ripe	2×100	94	88	83	Molded	87	82
Hollow Crown no. 23266.	Ripe	2×100	.....	95	94	Molded	.....	94
Hollow Crown unselected.	Ripe	4×100	.....	95	.....	.....	.....	.....
Hollow Crown no. 24702.	Green	2×100	70	35	65	Molded	.....	73
Hollow Crown no. 24266.	Green	2×100	72	77	68	Molded	70	79

constant or alternating temperatures between 15° and 27° C., while 32° C. is too high for both kinds of seeds.

Simultaneously with the germination tests, moisture determinations were conducted, the results of which are given in table II. They show that in each of the samples tested the green seeds contain a greater amount of hygroscopic moisture than the ripe seeds.

In an attempt to improve the germination of green seeds by lowering their water content to that of ripe seeds, different lots of unripe seeds were dried in various ways (table III). From the data in table III one may draw the following conclusions: (1) Germination of green seeds can be favorably affected by drying in a vacuum oven at 60° C. for four days. This result was confirmed by similar tests with six more series of samples. The average improvement of germination of dried over undried seeds was 16.6 per cent. (2) There is no

direct relationship between water content and germination quality, since all green seeds with improved germination have a very much lower water content than ripe seeds that germinate equally well. (3)

TABLE II

MOISTURE CONTENT OF FRESHLY HARVESTED, AIR-DRY PARSNIP SEEDS; NUMBERS REPRESENT AVERAGE OF THREE TESTS

METHOD OF DRYING	NO. OF SAMPLE	AMOUNT OF HYGROSCOPIC MOISTURE IN PERCENTAGE OF DRY WEIGHT	
		Ripe seeds	Green seeds
Vacuum 72° C.	24702	6.33	7.46
Vacuum 72° C.	24266	6.13	6.50
Access of air 94° C.	Guernsey	6.83	6.90

The method of drying is of importance. Drying at room temperature or temperatures between 72° and 85° C. does not affect the germination quality either favorably or unfavorably, while at temperatures above these a marked injury is noticeable.

TABLE III

GERMINATION OF GREEN PARSNIP SEEDS DRIED IN VARIOUS WAYS; NUMBERS REPRESENT AVERAGES OF FIVE TESTS WITH 100 SEEDS EACH

DRY TEMPERATURE	ORIGINAL MOISTURE CONTENT	LOSS OF MOISTURE	FINAL MOISTURE CONTENT	PERCENTAGE GERMINATION AT 20° C.
Undried seeds, fresh	7.4	.....	7.4	72.0
Seeds kept at room temperature for 4 months in paper bag	7.4	2.8	4.6	64.2
Fresh seeds dried at 60° C. in vacuum oven for 4 days	7.5	5.6	1.9	83.0
Fresh seeds dried at 72° C. in vacuum oven for 4 days	6.8	7.0	1.8	74.0
Fresh seeds dried at 90° C. with access of air 4 hours	7.4	6.1	1.3	78.0
Fresh seeds dried at 95° C. with access of air 4 hours	6.5	5.6	0.9	23.0
Fresh seeds dried at 103° C. with access of air 2 hours	6.8	4.2	2.6	51.0

This heat injury may be temporary only, provided the seed material is air-dry at the start and well ventilated during the drying. Table IV shows a slight temporary heat injury obtained in five sam-

ples of ripe seeds dried at 87° C. The seeds had completely recovered after one month. On the other hand, a complete destruction of viability occurs even at the optimum drying temperature of 60° C. when the samples are kept in sealed containers while they are heated.

After the percentage of vital seeds in freshly harvested parsnips and the optimum temperature or temperature range for germination had been found, storage experiments were set up in order to determine the influence of humidity, temperature, and ventilation upon the viability of stored seeds, and to find an improved method of storage. The seeds were arranged in the following series: (1) Air-dry seeds of known moisture content were stored in paper bags at

TABLE IV

EFFECT OF HIGH TEMPERATURES ON PARSNIP SEEDS KEPT IN OPEN AND IN CLOSED CONTAINERS; NUMBERS REPRESENT AVERAGE OF FIVE TESTS WITH 100 SEEDS EACH

MATERIAL	DRYING TEMPERATURE	CONDITION OF SAMPLE	GERMINATION AT 20° C.	
			Immediately	One month later
Hollow Crown Guernsey, ripe	87° C. 4½ hours	Weighing bottle uncovered	84.0	96.0
	87° C. 4½ hours	Weighing bottle sealed	None	None
	60° C. 4½ hours	Weighing bottle uncovered	96.7	95.8
	60° C. 4½ hours	Weighing bottle sealed	None	None

room temperature and in an ice chest (5°–7° C.); these seeds were to serve as controls for the following samples. (2) Air-dry seeds of known moisture content were stored in tightly stoppered bottles at 25° and at 5° C. (3) Seeds, the moisture content of which had been reduced to different degrees at various high temperatures, either with access of air or in vacuo, were stored in tightly stoppered bottles at 25° and 5° C. (4) Seeds were treated similarly to those mentioned under (2) and (3), but stored at temperatures between and below those recorded there. This last series is still in progress and will be discussed in a later paper.

When samples of seeds were taken out of the different storage conditions three years later, it was found that the temperature requirements for germination had changed. Green seeds from storage at ice box temperature showed a definite optimum germination at

15° C., instead of growing equally well at constant and alternating temperatures between 15° and 27° C. (table I). Samples of no. 24266, for instance, which had been stored in a paper bag in the ice chest, showed an average germination of 84 per cent at 15° C., 74 per cent at 20° C., and only 58 per cent at 15°–25° C. Green seeds stored at room temperature were all dead, so that all tests remained without results.

Ripe seeds had retained part of their vitality in all storage conditions. Table V shows how the optimum germination temperature had changed in various lots. All samples which had retained a high

TABLE V

CHANGE IN TEMPERATURE REQUIREMENTS FOR GERMINATION OF RIPE PARSNIP SEEDS DURING STORAGE

MATERIAL	WATER CONTENT	METHOD OF STORAGE	OPTIMUM GERMINATION TEMPERATURE (°C.)
Ripe, fresh.....	6.33 and 6.13	.....	15 or 20
Ripe, 3 years old.	Fluctuating 6.33–5.60	Room temperature, paper bag	15–25 alt.
Ripe, 3 years old.	Constant 6.13 and 6.83	Room temperature, sealed bottle	15–25 alt.
Ripe, 3 years old.	Reduced to various degrees, constant	Room temperature, sealed bottle	15
Ripe, 3 years old.	Fluctuating 6.33–9.10	Ice chest, paper bag	15
Ripe, 3 years old.	Constant 6.13 and 6.83	5° C., sealed bottle	15
Ripe, 3 years old.	Reduced to various degrees, constant	5° C., sealed bottle	15

percentage of vitality (as may be seen from tables VI and VII) germinated best at 15° C., while seeds of lower vitality (tables VI and VII) needed alternation of temperature.

Tables VI–VIII show the decrease in germination of the various samples under different storage conditions during the first three years of storage.

The effect of storing seeds in paper bags where they are exposed to variations in atmospheric humidity and temperature is shown in table VI. Ripe seeds retained their viability much better when stored in an ice chest as compared with laboratory storage, in spite of the high humidity in the ice box, which increased the moisture content of the seed considerably. Green seeds lost their vitality faster than ripe seeds under the same cool and humid conditions.

A storage of air-dry seeds in sealed containers under similar

temperature conditions proved very unfavorable at high as well as at cool temperatures (table VII), which shows that fluctuations in

TABLE VI

LOSS OF VITALITY IN VARIOUS COLLECTIONS OF PARSNIP SEEDS DURING STORAGE AT ROOM TEMPERATURE AND IN ICE CHEST IN PAPER BAGS

COLLECTION	NO. OF SEEDS USED	MOISTURE CONTENT	LENGTH OF STORAGE PERIOD	STORAGE TEMPERATUTE	PERCENTAGE GERMINATION	AT OPTIMUM GERMINATION TEMPERATURE (°C.)	PERCENTAGE LOSS OF VITALITY
Hollow Crown ripe, no. 24202, 1924	2×100	6.33	Fresh	.....	94.0	20	None
	3×100	5.60	2 years	Room temperature	72.3	20	20.6
	4×100	5.60	3 years	Room temperature	58.0	15–25	39.3
Hollow Crown ripe, no. 25266, 1924	2×100	6.13	Fresh	.....	95.0	20	None
	3×100	9.10	2 years	Ice chest	87.0	20	8.5
	4×100	9.10	3 years	Ice chest	84.0	15	11.6
Hollow Crown green, no. 24266, 1924	2×100	6.5	Fresh	.....	77.0	20	None
	3×100	8.8	2 years	Ice chest	54.0	20	29.9
	4×100	8.8	3 years	Ice chest	46.0	15	40.4
Hollow Crown unselected, 1925	2×100	6.8	Fresh	.....	94.0	20	None
	3×100	8.6	1 year	Ice chest	90.3	20	4.0
	4×100	8.6	2 years	Ice chest	78.0	15	17.1

TABLE VII

KEEPING QUALITY OF PARSNIP SEEDS WITH ORIGINAL WATER CONTENT STORED IN TIGHTLY STOPPERED BOTTLES AT VARIOUS TEMPERATURES; 4×100 SEEDS USED IN EACH GERMINATION TEST

MATERIAL DESCRIPTION	LENGTH OF STORAGE PERIOD	STORAGE TEMPERATURE (°C.)	PERCENTAGE GERMINATION	AT OPTIMUM GERMINATION TEMPERATURE (°C.)	PERCENTAGE LOSS OF VITALITY (°C.)
Hollow Crown ripe, no. 24702, 1924 moisture content 6.33%	Fresh	.....	88.0	20	.....
	2 years	25	22.0	20	75
	3 years	25	None	.....	100
Hollow Crown ripe, no. 24266, 1924 moisture content 6.13%	Fresh	.....	95.0	20	.....
	6 months	25	97.0	20	None
	2 years	25	60.7	20	37.5
	3 years	25	48.0	15–32	50.6
	3 years	5	54.0	15	47.4
Hollow Crown green, no. 24702, 1924 moisture content 7.46%	Fresh	.....	35.0	20	.....
	2 years	25	1.7	.....	95.2
	3 years	25	None	.....	100
	3 years	5	None	.....	100
Hollow Crown ripe, Guernsey, 1924 moisture content 6.83%	Fresh	.....	95.0	20	.....
	2 years	25	75.0	20	20
	3 years	25	10.0	15–25	85
	3 years	5	12.0	15	83

humidity and constant atmospheric moisture are less injurious to seed vitality than lack of ventilation for seeds of ordinary moisture content. There is an indication that the critical moisture content found by HEINRICH (2) for his seeds is somewhat below 6.13 per cent

TABLE VIII

KEEPING QUALITY OF SEEDS WITH REDUCED MOISTURE CONTENT STORED IN TIGHTLY STOPPERED BOTTLES AT VARIOUS TEMPERATURES; 4×100 SEEDS USED FOR EACH GERMINATION TEST

DESCRIPTION OF MATERIAL	TREATMENT	MOISTURE CONTENT	LENGTH OF STORAGE PERIOD	STORAGE TEMPERATURE (°C.)	PERCENTAGE GERMINATION	OPTIMUM GERMINATION TEMPERATURE (°C.)	PERCENTAGE LOSS OF VITALITY
Hollow Crown no. 24266, 1924 green	Dried 4 hours 90° C.	1.27	None	.....	83.0	20	None
		1.27	2 years	25	67.5	20	18.7
		1.27	3 years	25	59.0	15	29
		0.60	3 years	5	70.0	15	15.7
	Dried 24 hours 72° C. in vacuo	1.7	None	.....	61.0	20	None
		1.7	2 years	25	76.0	20	Improved
		1.7	3 years	25	75.0	15	Improved
Hollow Crown ripe, Guernsey 1924.....	Dried 1 hour 92° C.	1.25	None	.....	60.0	20	None
		1.25	2 years	25	51.0	20	15
		1.25	3 years	25	39.5	15	34.2
		1.60	3 years	5	47.5	15	20.8
	Dried 2 hours 92° C.	0.40	None	.....	80.0	20	None
		0.40	2 years	25	69.0	20	13.8
		0.40	3 years	25	50.0	15	37.5
		0.60	3 years	5	77.0	15	3.8
	Dried 4 hours 92° C.	0.40	None	.....	84.0	20	None
		0.40	2 years	25	75.5	20	10
		0.40	3 years	25	64.0	15	23.8
	Dried 6 hours 92° C.	0.50	None	.....	71.5	20	None
		0.50	2 years	25	73.0	20	None
		0.50	3 years	25	69.0	15	None
		0.10	3 years	5	71.0	15	None

in ripe parsnip seeds, since seeds with this moisture content keep almost as well as artificially dried seeds of a much lower water content. The keeping quality decreases rapidly, however, with an increase of hygroscopic moisture above 6.13 per cent. Again, green seeds show a greater sensitivity than ripe seeds.

The data in table VIII show that the unfavorable influence of lack of ventilation is avoided if the water content of the seeds is reduced by artificial drying. For green seeds the last method of

storage seems to be the only favorable one. It excludes fluctuating and high atmospheric humidities, to which green seeds have been shown previously to be very sensitive.

The fact that ripe seeds also retain their viability better when

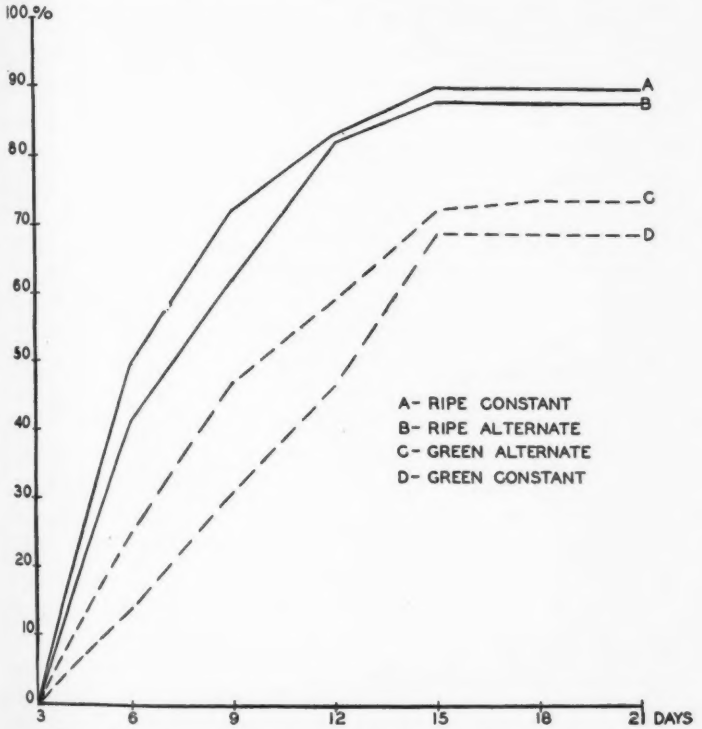

FIG. 1.—Rate of germination in ripe and green parsnip seeds at constant and alternating temperatures immediately after harvest.

stored artificially dried under exclusion of air is shown by a comparison of data in tables VII and VIII.

In addition to the loss in percentage of germination and a shifting in the optimum germination temperature, a slower rate of germination can be noted in stored seeds as compared with freshly harvested materials (figs. 1 and 2).

The germination rate in fresh seeds is highest during the first nine

days and decreases slightly during the following days. Germination is complete after the first fifteen days. The curves for green and ripe seeds are similar in shape, and those for alternating and for constant

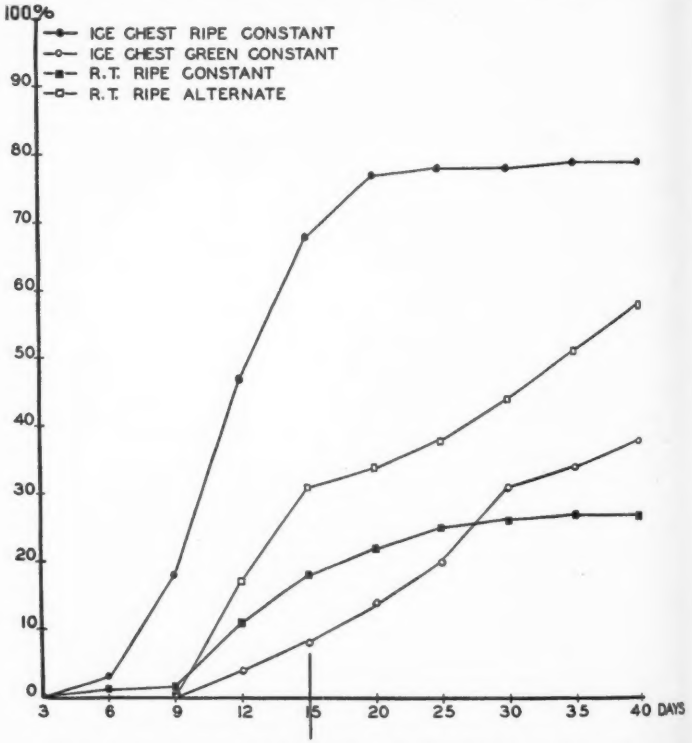

FIG. 2.—Rate of germination in parsnip seeds after three years of storage at room temperature and in ice chest with access of air.

temperatures run close together in both kinds of seeds. After three years of storage, however, curves plotted from similar tests have a very different form. For the first six to nine days the rate of germination is very low, but rises considerably during the following ten days. In seeds of well preserved vitality, such as ripe seeds stored in the ice chest, the optimum percentage of germination is almost reached at the end of this second period, which means that complete germina-

tion is reached after approximately twenty days, five days later than in freshly harvested seeds. In seeds of lower vitality, however, such as ripe seeds stored at room temperature or green seeds, germination proceeds slowly for more than one month, the highest percentage being reached only after forty days. It is also noticeable that the curves obtained from tests with stored seeds lack in regularity as compared with those obtained from experiments with new seeds. This may be due partly to infection by molds, to which stored seeds seem more susceptible than new seeds, but it may also be due to irregularity in absorption of water by stored seeds. In this respect the very low percentage of germination obtained in all stored seeds during the first six days is of special interest. Even seeds of high vitality, such as ripe seeds stored in the ice box, show this decrease in the rate of germination during the first few days. Later the factor which causes this retardation apparently is overcome, and the rest of the curve has the same shape as that for fresh seeds. In seeds of lower vitality, however, the rate remains low throughout the germination period.

Discussion

A comparison of the experimental results reported here with those obtained by MAQUENNE (3) shows that there are several ways of retaining vitality in parsnip seeds besides the one used by MAQUENNE. The writer was able to obtain almost equally good germination (94 and 90.3 per cent) in two consecutive years with parsnip samples of high quality stored under conditions which permit the continuation of metabolic processes (table VI). The writer has also been able to improve the vitality in green seeds during a period of three years (61–75 per cent germination) by storage at reduced moisture content after drying with artificial heat (table VIII). These storage conditions also did not exclude all life processes. It appears, therefore, that parsnip seeds can retain their vitality under certain storage conditions without being transferred to a state of "supermaturation" for at least as long a period as has been used by MAQUENNE in his experiments.

In agreement with the results of DUVEL (1), it was found that temperature and humidity are the main factors to be considered in

parsnip storage; that they are closely dependent upon each other in their effect on seed viability; and that humidity is the more important of the two.

These facts should be taken into account by nurserymen and foresters when they have to store short-lived seed material. At the present time seed growers suffer great losses from an annual discard of seeds which they are unable to sell after the first winter of storage, and foresters find it difficult to hold over a part of their harvest of short-lived fir and pine seeds from one year to another. Since most firs and pines are biennial or periodic bearers, and good harvests are therefore obtained only every second year or at longer periods, it is hard to find enough seed material of good quality for yearly plantings.

With a knowledge of the exact amount of moisture which may be retained in a seed at available storage temperatures without serious injury to seed vitality, it will be much easier for seed growers to store seeds with minimum loss of vitality for several years. For parsnip seeds this "critical moisture content" for ordinary room temperatures has been determined to lie below 6.13 per cent. With a hygroscopic moisture of more than 6.13 per cent the keeping quality of the seeds decreases markedly.

The main symptoms of devitalization, aside from a gradual loss in percentage of germination, are retardation in rate of germination and need of temperature alternations for germination. DUVEL, who has also noticed a decreasing rate of germination in his dry storage material, concludes that the seed coat must become impermeable to water and retard the absorption of moisture necessary to germination. This is not the case in parsnip, however, because the same retardation in germination rate can be observed when the dried seed coats are broken before the seeds are placed in the germination chamber.

Various other possible causes for slow devitalization in dry storage have been suggested by other workers, such as a colloidal rearrangement of substances in the embryo or a gradual denaturing of protoplasmic cell contents, but the experimental proof for these theories has not yet been obtained.

Summary

1. In germination tests conducted shortly after harvest, well-ripened brown seeds give better germination than green seeds.

2. The germination of green seeds can be improved through artificial drying at 60° C. in vacuo for four days.

3. There is no definite optimum germination temperature for freshly harvested seeds, constant and alternating temperatures between 20° and 27° C. being equally favorable.

4. With increasing age, parsnip seeds stored under unfavorable conditions require a temperature alternation of 15°–25° C. for germination, while seeds stored under conditions favorable to the retention of vitality germinate best at 15° C.

5. Stored in paper bags at room temperature, ripe parsnip seeds lose their vitality at a rate of 20 per cent during the first two years and approximately 60 per cent during three years of storage, when the moisture content of air-dry seeds is 6.33 per cent in the beginning and 5.6 per cent at the end of the storage period.

6. There are various ways by which the keeping quality of seeds may be improved. At a temperature of 5°–7° C. in an ice box the seeds keep for a considerably longer period, although their moisture content increases in the moist atmosphere of an ice box to more than 1.5 times that of seeds stored at room temperature (9.1 as compared with 5.6 per cent).

7. If the seeds are dried carefully and thoroughly, either for twenty-four hours in vacuo at 72° C. or for four to six hours at 90° C., to a moisture content of 0.40 to 1.7 per cent, their viability remains high even if they are stored at room temperature. To insure a continuous low moisture content, seeds treated in this way have to be kept in air-tight storage. The "critical moisture content" of parsnip seeds lies somewhat below 6.13 per cent.

8. Seeds which are not artificially dried cannot be stored under exclusion of air without losing their vitality very rapidly. Although a low storage temperature retards the death rate of unaerated moist seeds, it does not remove the injurious effect of lack of ventilation.

9. In advising practical storage methods for parsnip seeds, three methods of storing are suggested: (1) Storage at ice box temperature (approximately 5° C.) with frequent stirring of the seeds to secure

good ventilation; with this way of handling, even a very high atmospheric humidity is of little importance to the keeping quality of the seed. (2) When a higher storage temperature has to be used, a thorough drying of the seeds (90° C. for four to six hours) and a subsequent air-tight storage in sealed containers insure good keeping quality. (3) Optimum keeping quality should be obtained when artificially dried seeds are stored air-tight at low temperatures.

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